

Alessandro Piccolo *Editor*

Carbon Sequestration in Agricultural Soils

A Multidisciplinary Approach to
Innovative Methods

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to Innovative Methods

 Springer

Editor

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Preface

As part of the international quest to reduce greenhouse gas emissions into the atmosphere and on recommendation of the Kyoto Protocol, this book highlights alternatives to current soil management practices for turning agricultural soils into sinks of organic carbon. While common agronomic practices are based on traditional knowledge of soil transformation processes, this book indicates that modern or progressive understanding of complex biological systems in the soil ecosystem may already be exploited to devise new soil management practices. Explored in this book is the recent paradigmatic change in the chemical understanding of soil humus which has prompted new mechanisms for the control of soil organic matter stability. These mechanisms may be significantly more efficient at sequestering carbon in soil than current agronomic practices.

The body of this book reports findings of two methods for soil carbon sequestration related to their application in agricultural field trials. These methods are definitively based on the innovative understanding of soil organic matter chemistry as supramolecular association of small molecules (1) the protection from mineralization of labile soil molecules by the hydrophobic domains present in humified mature compost amended to soils, (2) the in situ oxidative photopolymerization of soil organic matter molecules after soil spreading with a biomimetic water-soluble iron–porphyrin catalyst.

The first method, although innovative in its mechanistic application, may be well considered within the current accepted soil management practices which makes use of exogenous organic matter (EOM). The second method is based on a catalytic chemical technology that appears still foreign within the traditional agronomic approach, to both the farming world and most agricultural scientists.

In the experience of the Editor of this book, proposing the catalytic mechanism of carbon sequestration in agricultural soil to a scientific audience was hardly received positively. There is a general skepticism of the use of biomimetic catalysts in agricultural soils, perhaps because of the possible negative consequences on the biological soil quality and the reduced nutritional functions of soils, due to a restricted availability of soil humus for microbial transformation.

The criticism was a beneficial stimulus to scale up research ambitions from laboratory or glasshouse to fully-fledged field agronomic trials, through which not only the effectiveness of the soil carbon sequestration methods could be verified in practice, but also the concerns about the eco-toxicological, biological, biotechnological and nutritional effects of the catalytic soil treatment could be dissipated.

A multifaceted research project was presented to the Italian Ministry of Research (MIUR) within the strategic FISIR programme. The intention was to cover all possible aspects inherent in soil organic matter transformations in agricultural soils leading to enhanced soil carbon sequestration, while maintaining soil quality and the high levels of crop productivity required by the farming market. The project was titled “Metodi Sostenibili per il sequestro del carbonio organico nei suoli agrari. Valutazione degli effetti sulla qualità chimica, fisica, biologica ed agronomica dei suoli”, with the MESCOSAGR acronym. The project was approved, under the coordination of this Editor, and was funded with a total budget of 2.5 Mio Euro over a 3 years working span.

The MESCOSAGR project relied on the work of six research units belonging to six different Italian Universities. In particular the University of Napoli Federico II comprised: the group of Prof. Alessandro Piccolo, for the determination of carbon and nitrogen sequestration in all treated soils, as well as the molecular transformation of soil organic matter upon soil treatments; the group of Prof. Fabrizio Quaglietta Chiarandà, for the evaluation of the agronomic effects of treatments on soils of the University experimental farm (Torre Lama); the group of Prof. Giancarlo Moschetti for the microbiological aspects of all project's treated soils; the group of Prof. Amalia Virzo for the evaluation of soil biological quality and emissions of greenhouse gases from field soils; the group of Prof. Stefano Mazzoleni for the development of a new modelling approach to predict soil organic matter dynamics in agricultural soils. The University of Torino was represented by the group of Prof. Carlo Grignani who led the overall agronomic experiments and conducted field trials at the University experimental farm (Tetto Frati). Dr. Giuseppe Celano was the head of the group of the University of Basilicata that had been in charge of ^{13}C and ^{15}N isotopic measurements in soil samples and conducted agronomic experiments under sorghum at the experimental farm of Battipaglia. The University of Bari was present with the group of Prof. Pacifico Ruggiero for the evaluation of genetic diversity in samples from treated soils. The University of Reggio Calabria took care of microcosm experiments and measurements of plant activities under the supervision of Prof. Maurizio Badiani. The group of Prof. Attilio del Re of the Catholic University of Piacenza evaluated the eco-toxicological parameters in all projects's soil samples and managed field trials at the local University experimental farm.

This book thus reports the main research findings of the MESCOSAGR project and amply responds to the queries placed by the early critics of the innovative methods for carbon sequestration in soil. Briefly, the methods were able to fix a significantly larger amount of carbon than that possibly sequestered by traditional methods. Concomitant to such very positive project outcome, both proposed

methods did not significantly alter the productive, physical, chemical, and biological potentials of the treated soils.

Readers will find in this book data and results of their own interest, but they will also have the advantage of being able to cross reference with other interdisciplinary subjects, thereby receiving a complete picture of the effects of the new soil management methods and their potential for practical application in farm management. I am also sure that the most perceptive soil scientists will find in the book several hints for new confirmative experiments, further ground for speculating on more soil–plant–technology interactions and the possibility to develop new methods or applications.

Finally, I take the chance to thank all the scientific and administrative collaborators of the MESCOSAGR project who made it possible, despite the many logistic difficulties often encountered, in reaching the project's ambitious objectives.

Portici, Italy
November 2011

Alessandro Piccolo

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Chapter 1

The Nature of Soil Organic Matter and Innovative Soil Managements to Fight Global Changes and Maintain Agricultural Productivity

Alessandro Piccolo

Abstract A new era in soil management is emerging on the basis of the novel understanding of soil organic matter (SOM), as a noncovalent supramolecular association of small molecules surviving microbial degradation of plant and animal tissues. The recognition of such molecular nature of humus may have technological implications in agricultural soil management that are yet to be developed. Here we discuss the implications of the supramolecular structure of humus on innovative methods for carbon sequestration in agricultural soil. One method exploits the capacity of humified/hydrophobic matter, such as mature compost amended to soils, to protect from mineralization biolabile hydrophilic molecules rhizodeposited by crops. Another method is the use of biomimetic catalysts to be spread on soils to oxidatively photopolymerize SOM in situ. The formation of intermolecular covalent bonds among soil humic molecules increases the chemical energy required by microbes to mineralize SOM. Both methods were verified in their effectiveness in soil before the scaling up of their use on real field trials under agricultural crops.

1.1 Current Concepts and Technologies

A sustainable use of soil means its exploitation in a way and at a rate that preserves at the long term its multitude of functions and protects or improves its quality, thereby maintaining its potential to meet the likely needs and aspirations of present and future generations (Van-Camp et al. 2004).

Soil organic matter (SOM) plays a fundamental role in plant nutrients status; maintenance of soil functions; and release of CO₂, methane, and other gases in the atmosphere. Two factors influence SOM content: natural (climate, soil parent

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material, land cover and/or vegetation and topography) and human-induced factors (land use, management, and degradation). In nature, uniform moisture conditions and comparable vegetation, the average total organic matter and nitrogen increase regularly from two to three times for each 10°C fall in mean temperature (Buckman and Brady 1960). Conversely, cultivation significantly affects OM content of soil by exposing fresh topsoil to rapid surface drying and air oxidation. Therefore, organic compounds are released to the atmosphere as a result of their biotic and abiotic degradation, while soil aggregates concomitantly break down due to progressive mineralization of binding *humic* materials. Unless OM is maintained or quickly replenished, the soil system is in a state of degradation, leading eventually to unsustainability (World Bank 1993). For example, a decline in OM content is accompanied by a decrease in soil fertility and biodiversity, and loss of structure, which together exacerbate overall soil degradation.

The current rapid depletion of OM in soils under farm land makes them sources of organic carbon rather than sinks. Organic carbon sequestration in soils is a potential tool for reducing greenhouse gas (GHG) emissions. The potential contribution of the agricultural sector to tackling climate change issues is now being acknowledged both under a strategic (i.e., in policy making) and practical standpoint. The Kyoto Protocol highlights that carbon sequestration in agricultural soils by land management practices can contribute to mitigating climate change. For example, estimates for Europe indicate that organic carbon sequestration in farm soils can account for about 20% of the total reduction required during the first commitment period (8% reduction required between 2008 and 2012 from a 1990 base) (EU Soil Thematic Strategy 2004). The role of soil, both as an emitter and a sink for carbon, is particularly important in this context. At global scale, research indicates that the soil carbon pool of 2,500 billion ton includes about 1,550 billion ton of soil organic carbon, which is 3.3 times the size of the atmospheric pool (760 billion ton) and 4.5 times the size of the biotic pool (560 billion ton) (Lal 2004). Between 1850 and 1998, the emission from terrestrial ecosystems was 136 ± 55 billion ton. The latter includes 78 ± 12 billion ton from soil, of which about one-third is attributed to soil degradation and accelerated erosion and two-thirds to OM mineralization (IPCC 2000). The European Union endorsed the need to link soil sustainability and its role in mitigating climate change, by calling for “a robust approach to address the interaction between soil protection and climate change from the viewpoints of research, economy and rural development, so that policies in this areas are mutually supportive” (EC 2006).

Management options available to sequester carbon in cropland include reduced and zero tillage, set-aside, perennial crops and deep rooting crops, more efficient use of organic amendments, improved rotations, irrigation, bioenergy crops, intensification of organic farming, and conversion of arable land to grassland or woodland (Smith et al. 2000, 2008). Due to advances in weed control methods and farm machinery which allow many crops to be grown with minimum tillage (reduced tillage) or without tillage (no till), these practices, which limit soil disturbance and consequently soil C losses through reduced microbial decomposition, are now usually believed to increase SOC sequestration in cropland soils. However, there

are no solid scientific bases to justify this belief (Cerri et al. 2004; Smith and Conen 2004; Gregorich et al. 2005; Plaza-Bonilla et al. 2010; Mancinelli et al. 2010). Moreover, a long-term application is usually required for reduced tillage practices to produce a significant and steady improvement of OC content in cultivated soils (West and Post 2002). Reduced tillage is also advocated to affect N_2O emissions but the net effect is inconsistent and depends on soil and climatic conditions (Marland et al. 2004). Additionally, the reduced tillage practices do not ensure a persistent organic carbon sequestration, since, as tillage is resumed (possibly by lack of sufficient incentives to farmers), the fixed carbon is rapidly lost again from soil. In fact, the incorporation of biolabile components derived from plant material is limited to soil surface (Six et al. 2000; Jacobs et al. 2010; Mishra et al. 2010), and their rapid decomposition is accelerated, if soil management is reversed to conventional tillage. Carbon sequestration in cropland by adopting reduced-tillage practices has been estimated (Fig. 1.1) to be rather small ($<0.5 \text{ ton C ha}^{-1} \text{ year}^{-1}$) and extremely variable ($>50\%$ error), thereby showing their little use in off-setting GHG emissions in Europe (Freibauer et al. 2004; Smith et al. 2007).

The shortcomings of current management practices for soil carbon sequestration based on reduced tillage are (1) reduced crop productivity; (2) small, inconsistent and variable carbon fixation; (3) temporary sequestration until traditional tillage practice is resumed. These shortcomings clarify that reduced- or no-till agriculture do not consistently result in soil C and N gain, and, in addition, it is not well quantified globally. Therefore, there is a clear and unmet need to find better alternatives to current soil management practices for organic carbon sequestration in agriculture.

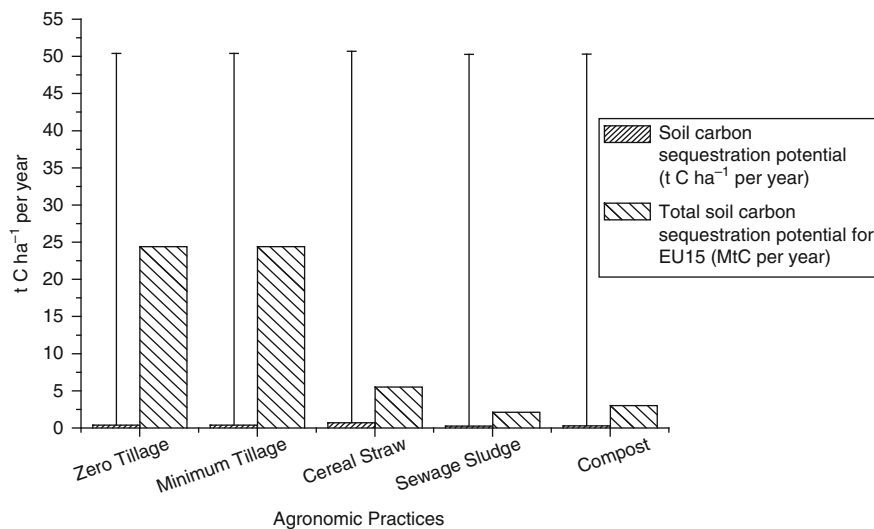


Fig. 1.1 Carbon sequestration potentials limited only by availability of land, biological resources and land suitability, and the potentials estimated to be realistically achievable by 2012

1.2 Conceptual Innovations

To meet the described need, it is required to introduce in agriculture more scientifically reliable, effective, and persistent soil management practices for carbon sequestration in soil. For the overall societal benefit, it is necessary to fully develop new technologies in agriculture with the added value given by advanced science that goes beyond traditional views.

An important feature involved in the stabilization and accumulation of OC in soils and sediments is the quality of organic matter. While SOC accumulation has been conceptually regarded as the saturation process of soil minerals by OC (Hassink and Whitmore 1997; Six et al. 2002), and, hence, governed by the physical control on decomposition (Scott et al. 1996; von Lützow et al. 2006), the OM chemical quality has been often considered as a secondary variable for SOC sequestration strategies. However, no direct or linear relation has ever been found between soil physical properties (texture, mineral composition, aggregation) and SOM stabilization (Dignac et al. 2002; Leifeld and Kögel-Knabner 2005).

In recent years, the concepts of C saturation in soil were further developed (Zhao et al. 2006; Stewart et al. 2009), and the relationship between the biochemical recalcitrance of humus and SOC stabilization processes has been taken in consideration (Augris et al. 1998; Lichtfouse et al. 1998; Kögel-Knabner 2002; Lorenz et al. 2007). In fact, the processes of SOM accumulation and decomposition depend closely on the molecular characteristics of the organic matter reaching the soil. This affects not only the amount of OM incorporated in soil, but also its chemical reactivity that regulates the function of OC pools as source or sink of atmospheric CO₂ (Baldock et al. 1992, 1997; Webster et al. 2000).

1.2.1 *The Supramolecular Structure of SOM*

A major breakthrough in understanding SOM chemistry in the last decade came with the recognition that soil humus is a self-assembled supramolecular associations of small heterogeneous molecules held together mainly by weak hydrophobic linkages, rather than being composed of large molecular weight macropolymers (Piccolo 2001).

Humus, otherwise referred to as Humic Substances (HS), is the natural organic matter comprising up to 80% of SOM. Because of the beneficial effects that HS have on the physical, chemical, and biological properties of soil, their role in the soil environment is significantly greater than that attributed to their contribution to sustaining plant growth. The HS are recognized for their controlling both the fate of environmental pollutants and the chemistry of organic carbon in the global ecosystem (Piccolo 1996).

Despite their prominent importance, a better knowledge of the basic nature and reactivity of HS has been elusive for a long time because of their large chemical

heterogeneity and geographical variability. Because it is a mixture that originates randomly from the decay of plant tissues or microbial metabolism–catabolism or both, the chemistry of humus is not only of utmost complexity but also a function of the different general properties of the ecosystem in which it is formed: vegetation, climate, topography, etc. The tremendous task of advancing the knowledge of humic chemistry and its consequences to other environmental domains still lies ahead of us. It should be obvious, to a world that appreciates the potentials of genetic engineering based on an understanding of DNA structure, that accurate predictions of reactivities and development of related technologies can only be made when there is a basic knowledge of the chemical structure of the reacting molecules.

Piccolo summarized his and other authors' experiments supporting the supramolecular structure of humus in different reviews (Piccolo 2001, 2002; Piccolo et al. 2003). These experimental results cannot be explained by analytical interferences or the traditional macropolymeric model of HS. They can rather be interpreted with the concept of loosely bound humic supramolecular associations. By this concept one can imagine HS as relatively small and heterogeneous molecules of various origin which self-organize in supramolecular conformations. Humic superstructures of relatively small molecules are not associated by covalent bonds but stabilized only by weak forces such as dispersive hydrophobic interactions (van der Waals, π – π , and CH– π bondings) and hydrogen bonds, the latter being progressively more important at low pHs. Hydrophilic and hydrophobic domains of humic molecules can be contiguous to or contained in each other and, with hydration water, form apparently large molecular size associations. In humic supramolecular organizations, the intermolecular forces determine the conformational structure of HS, and the complexity of the multiple noncovalent interactions controls their environmental reactivity.

By the concept of supramolecular association, the classical definitions of humic and fulvic acids are reconsidered. Fulvic acids may be regarded as associations of small hydrophilic molecules in which there are enough acidic functional groups to keep the fulvic clusters dispersed in solution at any pH. Humic acids are made by associations of predominantly hydrophobic compounds (polymethylenic chains, fatty acids, phenolic and steroid compounds) which are stabilized at neutral pH by hydrophobic dispersive forces (van der Waals, π – π , and CH– π bondings). Their conformations grow progressively in size when intermolecular hydrogen bondings are increasingly formed at lower pHs, until they flocculate.

1.2.2 The Conformational Flexibility of Humic Supramolecular Structures

The energetic implications behind the supramolecular structure of SOM are well depicted by molecular simulations through conformational softwares.

A minimization of conformational energy was conducted using HyperchemT 4.0 software to describe the interactions of humic supramolecular associations with an organic acid. Eleven different molecular structures of compounds identified as components of HS (Stevenson 1994) were grouped together in the simulation to form a supramolecular association. The structures represented molecules such as saturated and unsaturated fatty acids, carbohydrates, peptides, lignin derivatives, etc., with molecular weights varying from 116 Da for a dihydroxybenzene to 504 Da for a triglucose. The molecular weight sum of the 11 molecules was 3,065 Da.

The geometry of the association was automatically adjusted and its conformational energy was minimized in the vacuum (Fig. 1.2a). Ten molecules of acetic acid were added first to surround the hypothetical supramolecular association (Fig. 1.2b) and then placed within the conformation of the association (Fig. 1.2c). The resulting association energies were calculated by the software to be 114, 91.2, and 84.0 Kcal mol⁻¹, respectively.

The association of the different molecules also varied its physical appearance with the approach of acetic acid molecules which caused a loosening of intermolecular attractions until some spaces among the molecules were formed.

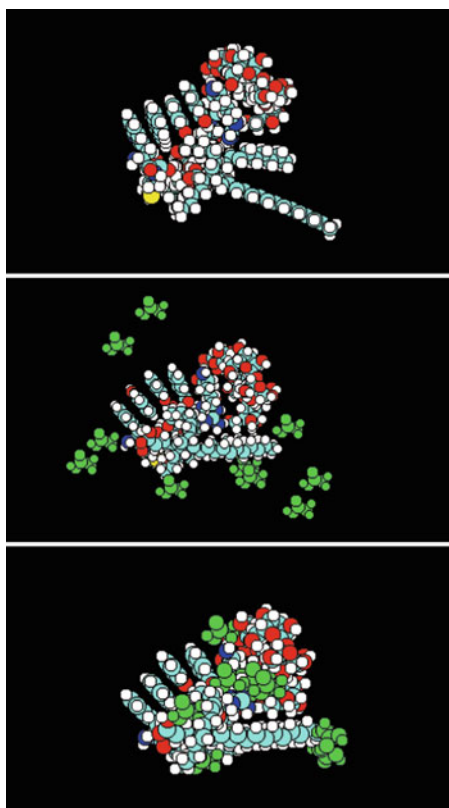


Fig. 1.2 Computer simulation of the optimum conformational energy (in vacuo) for an association of 11 different humic precursors with a total molecular weight of 3,065 Da. (a) *Upper picture*: molecular association with an energy of 114 Kcal mol⁻¹; (b) *middle picture*: molecular association surrounded by ten molecules of acetic acid with an energy of 91.2 Kcal mol⁻¹; (c) *lower picture*: molecular association containing ten molecules of acetic acid with an energy of 84.0 Kcal mol⁻¹

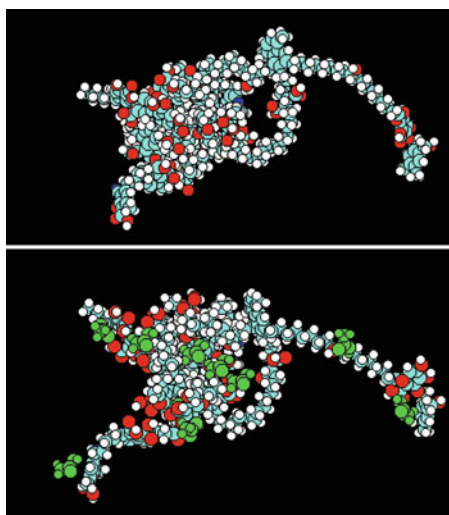
The computer simulation (Fig. 1.2) pictorially shows that the addition of organic acids to humic molecules is capable of reducing the solvation energy and, concomitantly, causing a partial disruption of their association. These results are in line with the different experiments describing the behavior of organic matter dissolved in solution reported by Piccolo (2001, 2002).

Conversely, when a covalently linked structure of HS, based on the traditional macro polymeric model, was placed in the same exercise of molecular simulation, no significant changes in energy content and physical association were noted with the addition of acetic acid. Figure 1.3a shows that a covalently bonded polymeric structure with a molecular weight of 6,326 Da is not significantly altered (Fig. 1.3b) by the same number of acetic acid molecules used for the simulation of the weakly bound supramolecular association shown in Fig. 1.2. Moreover, the gain in conformational energy was only of 10 Kcal mol^{-1} , passing from $627.40 \text{ Kcal mol}^{-1}$ for the polymer to $617.26 \text{ Kcal mol}^{-1}$ for the same polymer added with acetic acid. Thus, it would be hardly possible, using this hypothetical polymeric model, that the simple addition of acetic acid molecules to such a high molecular weight polymer would provide a rearrangement of molecular associations leading to conformational disruptions as that described by the experiments funding the supramolecular structure of SOM (Piccolo 2001, 2002).

1.3 Implications in Soil of the Supramolecular Structure of Humus

A clarification of the aggregate structures of HS has represented a major innovation in humus chemistry. A model of soil humus as a supramolecular association of small molecules, originated from extended microbial degradation of different plant

Fig. 1.3 Computer simulation of the optimum conformational energy (in vacuo) of a hypothetical covalently linked humic polymer (MW = 6,326 Da) as hypothesized by Stevenson (1994). (a) *Upper picture*: humic polymer having an energy of $627.40 \text{ Kcal mol}^{-1}$; (b) *lower picture*: humic polymer containing ten molecules of acetic acid and having an energy of $617.26 \text{ Kcal mol}^{-1}$



and animal biomolecules and assembled together by mainly hydrophobic forces strengthened by the hydrophobic effect, may well have implications on how we regard the phenomena of accumulation and decomposition of SOM. In fact, the new consensus on the supramolecular structure of HS has several implications for the control soil OM (Piccolo 2011, 2002).

1.3.1 The Concept of Humification

It has been increasingly proved that simple, mainly alkyl, recalcitrant organic compounds deriving from both plant residue decomposition and microbial resynthesis are progressively incorporated into the most stable SOM fractions (Piccolo 1996; Lichtfouse 1998). Piccolo (1996) proposed that hydrophobic humic components in soil protect easily degradable compounds. He postulated that incorporation of polar molecules in associations of hydrophobic components may contribute to prevent an otherwise rapid microbial degradation of hydrophilic molecules and enhance their persistence in soil. This hypothesis is in accordance with the model of humic superstructures, by which humic molecules self-assemble into hydrophobic or hydrophilic domains according to their reciprocal affinity.

Based on this, the concept of humification must be revised or corrected. Humification should not be longer intended as increased polymerization of soil organic compounds, as previously assumed, but as a progressive accumulation of hydrophobic and recalcitrant relatively small humic molecules in superstructures (Piccolo 1996; Lorenz et al. 2007). However, the heterogeneity of humic molecules in soil leads to the formation of mixed supramolecular structures. It was shown that the stable soil humic superstructures still contained hydrophilic molecules encapsulated in the hydrophobic domains, thereby being protected from biological degradation (Xu and Hatcher 2002; Piccolo et al. 2005a; Spaccini et al. 2006). This phenomenon was interpreted as a mechanism of hydrophobic protection by which labile hydrophilic molecules are included in hydrophobic humic superstructures and preserved from mineralization. It could be thus assumed that tightly bound humic associations containing mainly resistant alkyl remains of vegetative tissues may incorporate, by a random self-organizing process, also a few hydrophilic molecules or associated clusters of them.

1.3.2 The Mechanism of Hydrophobic Protection of SOM to Sequester Carbon in Soil

The recognized importance of hydrophobicity in stable SOM has a relevant implication in soil carbon sequestration. In fact, the hydrophobic character of OM represents a biochemical hindrance to microbial decomposition (Piccolo et al. 1999;

Spaccini et al. 2000), the basis for a persistent soil aggregate stability (Piccolo and Mbagwu 1999), and an overall SOM stabilization (Rumpel et al. 2004; Winkler et al. 2005; Zhou et al. 2010). The recalcitrant hydrophobic molecules are the constituents of the stable and humified SOM fraction (Piccolo 1996; Grasset et al. 2002; Deport et al. 2006), that enters in intimate association with fine soil particles, such as clay minerals and Fe and Al hydroxides, thus contributing to highly stabilize soil organo-mineral complexes (Mikutta et al. 2006; Schöning and Kögel-Knabner 2006; von Lützow et al. 2006).

Furthermore, the porous architecture of hydrophobic domains of soil humus exerts a dynamic mechanism of hydrophobic protection toward the biolabile organic compounds released in soil solution by plant roots exudates and microbial degradation of crop biomolecules. It was experimentally verified by measuring the reduced degradation of ^{13}C -labeled compounds in soils amended with humified matter at different degree of hydrophobicity (Spaccini et al. 2002). These authors synthesized a ^{13}C -labeled 2-decanol as a model of an easily degradable molecule in soil. They partitioned the labeled molecule into solutions of two humic acids, one from compost (HA-C) and one from lignite (HA-L), of different degrees of hydrophobicity. The two labeled humic solutions and one solution containing only the labeled 2-decanol (soil + ^{13}C) were added to a soil and incubated at field capacity for 3 months. The treated samples and a control soil were sampled periodically and the ^{13}C content was measured by high-resolution mass spectrometry. It was found that the biolabile ^{13}C -labeled 2-decanol was protected from mineralization when incorporated into the hydrophobic domains of the HS. The highly hydrophobic and more aromatic humic acid from lignite was more effective than the one from compost in sequestering the carbon from 2-decanol. After incubation, the residual ^{13}C -labeled OC recovered in bulk soil was equal to 28, 45, and 58% of the original content for samples containing the labeled alcohol alone or with HA from compost and lignite, respectively.

The same experiment by Spaccini et al. (2002) also followed the ^{13}C -OC distribution in the particle-size fractions of the treated samples. The residual ^{13}C -OC among soil particle sizes indicated that the hydrophobic protection was most effective in the silt- and clay-sized fractions (Fig. 1.4). This result confirms the importance of associations between fine textural fractions and microbially recalcitrant OM and suggests that SOM accumulation due to hydrophobic protection preferentially occurs within organo-mineral association of finer soil particles. Nevertheless, hydrophobic sequestration of carbon in soil may also take place within larger size fractions, provided that humified matter of large hydrophobic character is applied. In fact, the highly hydrophobic HA from lignite was able to reduce OC decomposition, with respect to treatments with HA from compost and ^{13}C -2-decanol alone, even in the coarser fractions which are commonly associated with rapid cycling of SOM pools.

Exogenous organic matter (EOM), such as mature compost added to soils, may also be capable of reducing the biological mineralization of labile polysaccharides due to progressive encapsulation into hydrophobic domains of compost. In a long-term (1 year) experiment, Piccolo et al. (2004) treated both a sandy and a silty-loamy

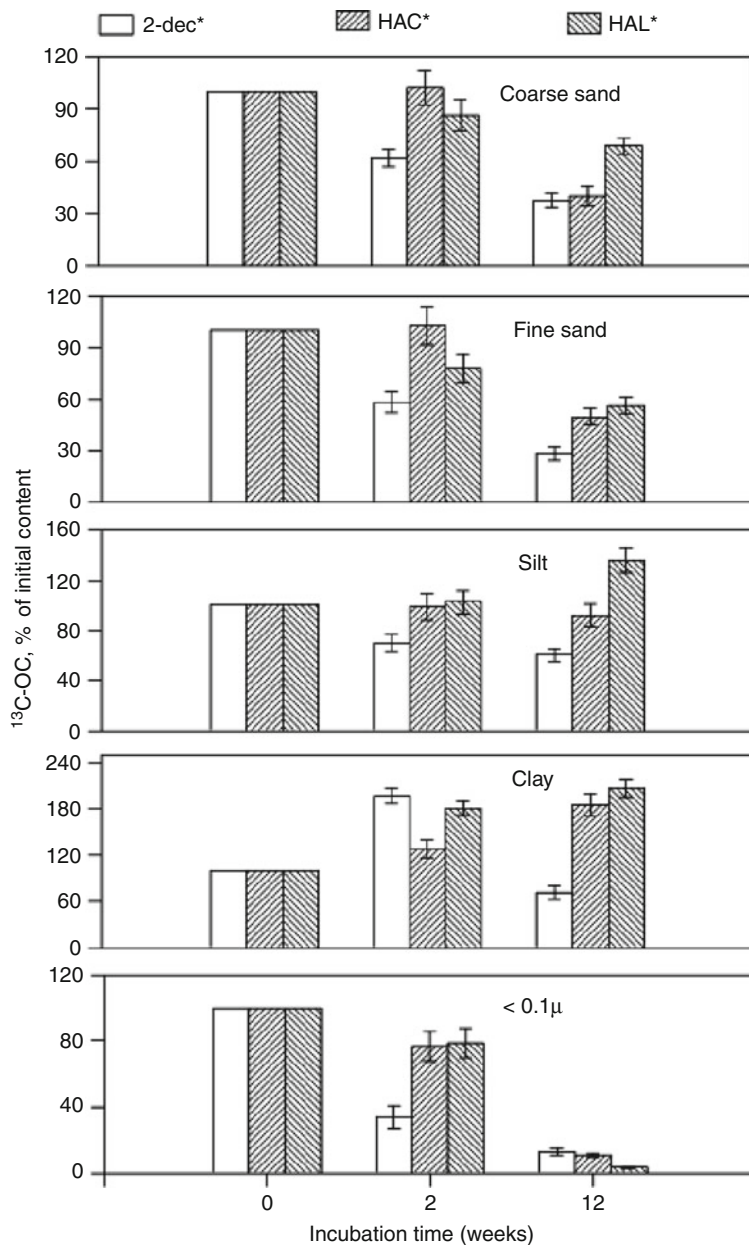


Fig. 1.4 Variation in comparison to time 0 of ^{13}C -SOM content in soil particle-size fractions according to treatments (^{13}C -2dec., treatment with only ^{13}C -labeled 2-decanol; ^{13}C -HAC, treatment of HA from compost previously added with ^{13}C -labeled 2-decanol; ^{13}C -HAL, treatment of HA from lignite previously added with ^{13}C -labeled 2-decanol). Bars in graph indicate standard deviation ($n = 3$)

soil with a mature compost before and after addition of a labile polysaccharide to verify whether compost was able to reduce mineralization of the biolabile material. Mature compost induced a significant reduction of OC losses in both soils, thus confirming that labile organic matter in soils can be protected from biodegradation by repartition into the hydrophobic domains of the stable, humified organic matter. This study suggests that mature compost and humic acids may usefully integrate management practices aimed to sequester organic carbon in soils.

Thus, amendments of mature compost to soil is expected not only to improve the quantitative and qualitative status of SOM (Adani et al. 2006; Shindo et al. 2006; Spaccini et al. 2009), but to also increase the content of humified and hydrophobic organic components (Spaccini and Piccolo 2007; Caricasole et al. 2011), which can contribute to reduced OC mineralization and turn the soil to be a sink of organic carbon (Spaccini et al. 2002; Fortuna et al. 2003; Piccolo et al. 2004; Lal 2009).

1.3.3 Increasing Microbial Stability of SOM by In Situ Catalyzed Polymerization of Humic Components

Understanding humus as a supramolecular association of small molecules means overcoming the limitations imposed by the paradigmatic polymeric model. In fact, another important implication of the supramolecular view of SOM resides in the fact that some self-assembled small soil humic molecules can then be coupled together into larger molecular weight materials, thereby increasing the amount of energy-rich intermolecular interactions (covalent bonds) in the superstructures. This would signify an enhancement of the energetic level that soil microbes must overcome to make use of the chemical energy needed for their own metabolism, thereby inhibiting their OM degradation capacity and consequent reduction of GHG emission.

The oligo-/poly-merization of humic heterogeneous molecules can be achieved by applying current chemical technologies based on oxidative catalysis promoted by specific enzymes (Piccolo et al. 2000; Peralta-Zamora et al. 2003). Piccolo (2002) showed that by subjecting some synthetic clay–humic complexes to an oxidative reaction with a peroxidase enzyme as a catalyst and H₂O as oxidant, the extraction of humic matter from the treated material was lower than from control. The yield of extraction from humic–clay complexes decreased by more than 40% after the oxidative coupling reaction. These results indicated that polymerization of humic molecules occurred also at the interphase of the solid clay–humic complexes, and the increase in molecular size of the humic materials was the most probable cause for the reduction in extraction yields. It seemed then possible to induce the polymerization of HS in natural soil samples in order to control or change the properties of native SOM.

However, the use of enzymes to promote oxidative coupling among humic molecules cannot be recommended as a field practice due to adsorption of enzymes on soil particles, and consequent denaturation and loss of catalytic activity.

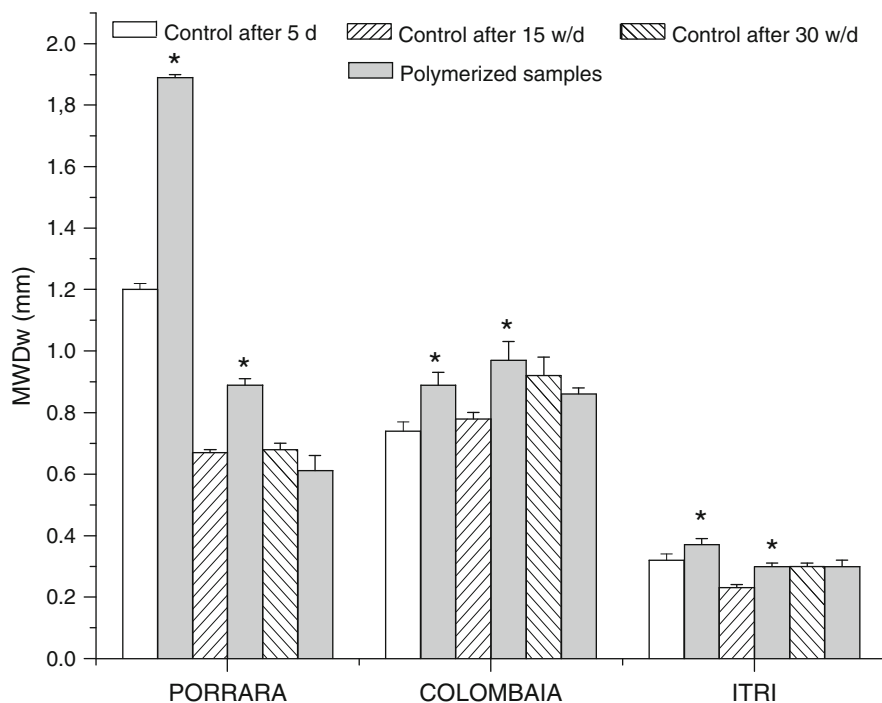


Fig. 1.5 Mean Weight Diameter in water (MWDw) for the three soils, Porrara, Colombaia, Itri, before and after the photo-polymerization treatment for 5 days incubation, and 15 and 30 wetting and drying cycles (w/d). Error bars indicate standard error ($n = 3$). The *asterisks* denote significant differences between control and treatment at the level of $P \leq 0.05$

Conversely, a biomimetic catalyst, such as biocompatible metal–porphyrins that mimic the activity of the *heme* prosthetic group of oxidative enzymes (e.g., laccase, peroxidase) without its cumbersome protein envelope, can be made adequately water soluble to maintain its catalytic activity in the soil environment (Sheldon 1994).

While soil treatment with mature compost is increasingly recognized as a valuable practice for SOM stabilization, the use of biomimetic catalyst represents an absolutely innovative technology to increase the SOC sequestration process. Humic molecules in solution were found to oxidatively polymerize and provide more rigid products under the catalysis of a water-soluble iron–porphyrin (Piccolo et al. 2005b). This polymerization reaction was shown to also occur under photo-oxidation without the need of a chemical oxidant (Šmejkalova and Piccolo 2005). The oligomers formed during the biomimetic catalyzed reaction were isolated and characterized by Šmejkalova et al. (2006, 2007). It was found that up to pentamers were formed during the oligomerization of phenolic precursors.

The results obtained on the polymerization of HS by both enzymatic and biomimetic catalysis encouraged to step further in the research. A laboratory incubation on three Mediterranean soils was conducted to verify the impact of an

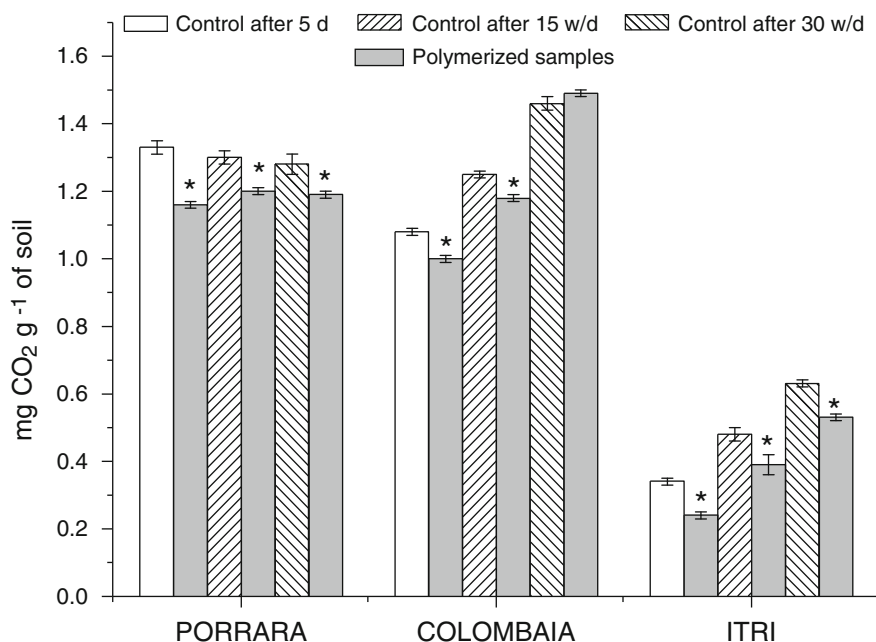


Fig. 1.6 Soil respiration ($\text{mg CO}_2 \text{ g}^{-1}$ of soil) from the three soils, Porrara, Colombaia, Itri, before and after photo-polymerization treatment for 5 days incubation, and 15 and 30 wetting and drying cycles (w/d). Error bars indicate standard error ($n = 3$). The asterisks denote significant differences between control and treatment at the level of $P \leq 0.05$

in situ photo-polymerization of soil OM catalyzed by the biomimetic iron–porphyrin under solar irradiation, on the physical status of the soils, their organic carbon distribution in soil particle sizes, and the reduction of microbial respiration (Piccolo et al. 2011). These soils were also subjected to long period of wetting and drying (w/d) to assess the capacity of the photo-polymerized SOM to sustain soil processes which affect both soil physical and biological quality. The in situ photo-polymerization reaction increased water stability of soil aggregates both after 5 days incubation and 15 w/d cycles, but not after 30 w/d cycles (Fig. 1.5).

The gain in soil physical quality was reflected by the shift of organic carbon content from small to large soil aggregates, thereby suggesting that the photo-polymerization stabilized OC by both chemical and physical processes. Finally, a further evidence that the photo-catalytic treatment enables an effective sequestration of carbon in soil was provided by the significant reduction of CO_2 respired by all soils after both 5 days incubation and w/d cycles (Fig. 1.6).

Both mechanisms of hydrophobic protection by humified matter such as compost and in situ catalyzed photo-polymerization of SOM received a solid scientific basis that pointed out how they were both effective in favoring carbon sequestration in soil. Both mechanisms were chemically and physical-chemically based and were far different than any other method so far attempted to increase the role of OC sink of soils.

1.4 Scaling Up Innovative Concepts into Agricultural Field Experiments

The described innovative concepts whose theoretical mechanism and functioning were derived from laboratory experiments, need not only to be verified in real field scale conditions, but also to be proved harmless to the physical, chemical, nutritional, and biomolecular quality of soils. This overall objective was looked for in the MESCOSAGR project.

At the basis of this project there was the intention to go beyond the traditional practices for carbon sequestration and put in practice the innovative concepts which stemmed from the described novel scientific understanding of OM chemistry and transformation in soil (Piccolo and Teshale 1998). Field experimentation was then scientifically conducted in four Italian agricultural sites located in experimental farms belonging to different universities (Fig. 1.7).

The details of the experimentations can be found in Chaps. 3, 5, and 7. The following two innovative technologies were thus adopted as soil treatments in the experimental sites under different crops and in comparison to other agronomic

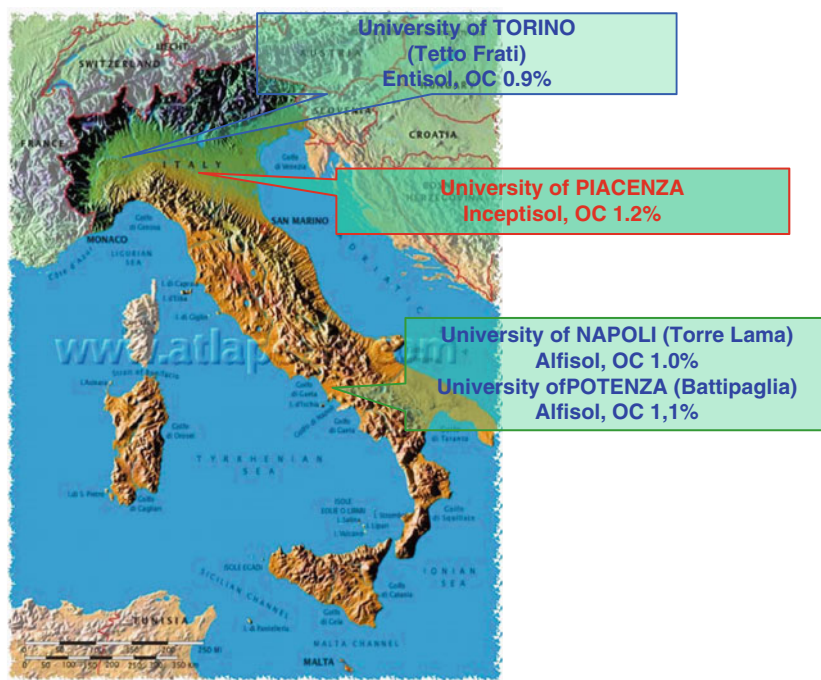


Fig. 1.7 Experimental sites of the MESCOSAGR project in Italy

practices for carbon sequestration. The soils had different textures and placed along a north-south transect in order to account for differences in climatic conditions:

1. Hydrophobic protection by humified mature compost. The general beneficial effects of humified compost on farm productivity (increase of soil fertility status and structural stability) were evaluated in combination with its potential to sequester the hydrophilic organic compounds released in soil during crop growth (microbial activity and plant exudates) into the humified hydrophobic superstructures of compost. While compost is progressively applied to agricultural soils within organic farming practices, very few data are present in the literature up to now on its capacity for a net sequestration of organic carbon in soil. Soils under maize were treated with two rates of mature compost (10 and 20 ton ha⁻¹ year⁻¹) and their carbon sequestration capacity compared with that of traditional and minimum tillage practices.
2. In situ photo-polymerization of soil organic molecules by biomimetic catalysis. This process is meant to provide enhancement of chemical energy in soil organic molecules, thereby reducing the metabolic capacity of soil microbes to degrade OM. This mechanism of chemical stabilization, joint to the consequent physical inaccessibility of OM, represents a persistent fixation of organic compounds in soil. The MESCOSAGR project was intended to field prove the validity of this innovative technology in order to introduce it as a routine agronomic practice to enhance carbon sequestration in agricultural soils. This goal may not only contribute to fulfill the recommendations of the Kyoto protocol, but also suggest the agro-industry that there is a possible way to develop new eco-compatible agrochemicals to be used in the issue of carbon sequestration in agriculture.

Both these innovative soil management practices proposed in the MESCOSAGR project should have the following advantages:

- Sequester carbon more efficiently and persistently than other methods, while maintaining crop productivity and farmers income
- Efficient organic farming by recycling biomass into mature compost
- Integrate advanced science and technology into agricultural practices
- Reduce GHG emissions from agriculture
- Maintain or even improve physical, chemical, and biological quality of treated soils
- Stimulate agrochemical technology and industry

Although the MESCOSAGR project is the first attempt of our knowledge to conjugate exogenous additions to soils with the aim of carbon sequestration and reduction of GHG, we are also aware that this will be not sufficient to promote the innovative methods to a larger scale of application. However, it is hoped that the example of MESCOSAGR will be followed by other field trails in Italy and in other countries.

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Chapter 2

The Kyoto Protocol and European and Italian Regulations in Agriculture

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Abstract Climate change represents the most important challenge for the international scientific community, for the inherent and irreversible modification brought about in natural ecosystems. International institutions increasingly adopt measures to promote preservation of ecosystems and counteract the social and economical consequences of environmental decline. Here we review the actions undertaken by both the Intergovernmental Panel for Climate Change (IPCC) and the United Nations Framework Convention on Climate Change (UNFCCC), aimed to stabilize and reduce concentrations of greenhouse gases (GHG) in atmosphere, including the Kyoto Protocol that obliges developed countries to provide the political and legal framework to meet the Protocol's expectations. Moreover, it is mandatory for national policies to reduce the occurrence of main risky events, such as landslides, floods, and desertification processes, whose frequency have rapidly risen in the Mediterranean regions mostly susceptible to climatic changes. According to the Kyoto Protocol, each signed party should include, in its annual GHG inventory, information on GHG possibly removed by means of carbon sinks activities such as land use, land-use change and forestry (LULUCF). Italian laws encompass the *National System for the Italian Greenhouse Gas Inventory* and the *National Registry for Carbon sinks*. The latter estimates GHG emissions by sources and accounts for their net removal based on sinks of the LULUCF sector that includes forest land, cropland, grassland, wetlands, and settlements. These compartments in 2008 removed 87.3 Mt of CO₂ from atmosphere while, from 1990 to 2008, the total removal as CO₂ equivalent increased by 34.8%, CO₂ accounting for more than 99% of both total emissions and removals of the sector. Within this frame, carbon

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sequestration in cultivated soils has become important to add new carbon sinks other than LULUCF. The relation of soil management practices to the increase in soil organic matter is a basic requirement to develop a solid methodology to assess carbon stock changes in soil pool and provide a useful database over the national territory.

2.1 The Climate Change as a Global Environmental Problem

The Intergovernmental Panel for Climate Change (IPCC) refers to climate change as an alteration in the mean and/or the variability of the climate that is identifiable (e.g., using statistical tests), and persistent for an extended time period, typically decades or longer. It refers to any change in climate over time, whether due to natural variability or to a result in human activity. This concept differs from that of the United Nations Framework Convention on Climate Change (UNFCCC), where climate change refers to an alteration of climate that is attributed directly or indirectly to human activity, that alters the composition of the global atmosphere, and it is observed over comparable time periods, in addition to natural climate variability (IPCC 2007a).

The first IPCC report submitted in 1990 confirmed the legacy of the climate change phenomenon with human activities, and essentially identified two main causes: the use of fossil fuels related to greenhouse gas (GHG) emissions and the reduction of principal reservoirs of carbon in the planet, especially forests. The report highlighted the need to develop a comprehensive strategy to tackle climate change and its consequences, drawing up the following key principles on which to base a possible international convention:

- The need to sensitize all countries on the global nature of phenomenon
- The use of an equity criterion in determining response actions
- The existence of responsibilities common to all countries, but differentiated by the degree of economic development of each country
- The precautionary principle, which states that the uncertainty of phenomena from the scientific point of view is not an excuse for not addressing the problem

In the same year, the General Assembly of the United Nations created the UNFCCC, adopted on May 9, 1992. The Convention entered into force on March 21, 1994. Currently, 194 parties (193 states and 1 regional economic integration organization) have signed the convention. The convention is complemented by the Kyoto Protocol which was adopted on December 11, 1997 and entered into force on February 16, 2005. Under this treaty, 37 industrialized countries and the European community have committed to reduce GHG emissions to an average of 5% of the 1990 levels over the 5-year period 2008–2012 (first commitment period). Up to date, 192 parties have ratified the treaty. The difference between the convention and the protocol is that the convention encourages industrialized countries to stabilize GHG emissions, while the protocol has the specific target to reduce GHG emissions.

The ultimate objective of UNFCCC is to stabilize concentrations of GHG in the atmosphere at a level that would prevent dangerous anthropogenic interference with

the climate system. Such a level should be achieved within a sufficient time frame to allow ecosystems to adapt naturally to climate change, ensure that food production is not threatened, and enable economic development to proceed in a sustainable manner.¹

The Kyoto Protocol is an international treaty on the environmental global warming, signed on December 11, 1997 by more than 160 countries at the UNFCCC conference. The treaty entered into force on February 16, 2005, following ratification by Russia. For the enforcement of the protocol, it was necessary that it be ratified by at least 55 signatory countries and that ratification generated at least 55% of all global emissions. Italy ratified the treaty with the Law 120 of June 1, 2002. The treaty provides the obligation of developed countries during 2008–2012 to reduce polluting emissions (carbon dioxide, methane, nitrous oxide, hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulfur hexafluoride) to no less than 5% of the emissions recorded in 1990, considered as the base year.

The Kyoto Protocol dictates that countries must meet their targets primarily through national measures. However, the protocol also offers to signatory countries three market-based mechanisms to meet their targets: Emission Trading, Clean Development Mechanism (CDM), and Joint Implementation (JI). Moreover, the protocol requires that countries' emissions need to be monitored and precise records on the trading process must be kept. A registry system tracks and records transactions among parties under different mechanisms. The UN Climate Change Secretariat keeps an international transaction logbook to verify that transactions are consistent with the rules of the protocol. Furthermore, reporting is periodically done by parties by submitting annual GHG emission inventories and national reports.

Article 3 of the Kyoto Protocol allows each signed party to include in its annual GHG inventory information about anthropogenic GHG emissions that may have been removed by means of sinks represented by land use, land-use change and forestry (LULUCF) activities. In particular, paragraph 3 of Article 3 is related to the activities of afforestation, reforestation, and deforestation, which had begun since 1990 and resulted from a direct human-induced conversion. In the context of the paragraph 3.4, each party may choose to account for anthropogenic GHG emissions removed by means of sinks resulting from any or all of the following human-induced activities: forest management, cropland management, grazing land management, and revegetation. Activities enclosed in Article 3.4 encompass lands that have not undergone conversion since 1990, but are otherwise subjected to a specific land use (UNFCCC 2005a, b).

The convention and the Kyoto Protocol are also designed to assist countries in adapting to the adverse effects of climate change. The convention facilitates the development and deployment of techniques that can help increase resilience to the impacts of climate change. For this purpose, the Adaptation Fund was established to finance adaptation projects and programmes in developing countries which have

¹ <http://unfccc.int/resource/docs/convkp/conveng.pdf>

become full parties of the protocol. The fund is financed mainly with a share of funds related to CDM project activities. Currently, negotiations for the second commitment period under the Kyoto Protocol are under way, and are oriented to more stringent emission reductions, as indicated by IPCC in their recent reports.

2.2 The Risks of Climate Change

The Fourth Assessment Report (FAR) from the IPCC (2007a) confirms that the consequences of climate change will not only be restricted to an increase in average planet temperature, but it will also entail the change of the entire climate system, including precipitation, wind, frequency and intensity of extreme events, having different impact in the diverse world regions. Studies carried out with global circulation models (GCM) and regional circulation models (RCM) with regard to Europe have both indicated the following:

- Increase in average temperature in Europe
- Decrease in rainfall in Southern Europe
- Increase in rainfall in Northern Europe
- Possible increase in the frequency of heavy rainfall events across Europe

The last point of this list highlights the risk of growing intensity of floods and landslides throughout the Italian territory. In fact, the climatological data indicate that while water scarcity will be a future problem issue for Italy, the possibility for occasional floods with more severe impact than the average of previous events should not be overlooked. Similar considerations apply to landslides, to which the Italian hilly and mountainous territory is particularly vulnerable and they can be triggered by heavy rainfall events (Bigano and Pauli 2007).

Data provided by Emergency Events Database (EM-DAT 2011) indicate that floods were the main type of natural disaster in Europe with 283 disastrous floods in the last 25 years, with an increasing frequency. In regard to Italy, data indicate that, among natural disasters, floods are ranked first, in terms of affected population, and second, in terms of economic damage. Landslides, being localized phenomenon, affect fewer people and cause less damage, but claim more victims than floods. The relevant EM-DAT statistics for Italy from 1900 to 2011 are summarized in Table 2.1. It is important that the impact of climate change is taken into account in policies aimed to protect from the geological risk.

Climate change may affect not only the quantitative status of water resources, but also its quality, due to alteration of the hydrological cycles. The main impacts on freshwater resources are the shifts in rain and snow cycles, changes in the availability and demand for water, variations in water quality, temperature and nutrient content, and fast melting down of glaciers with occurrence of flash floods (IPCC 2007b).

The relationship between landslides and climate change is very complex and less direct than the risk of flooding. It is clear that rain can cause landslides, with

Table 2.1 Major disasters in Italy from 1905 to 2006

Disaster	Type	Events number	Death casualties	Total affected	Damage (thousands US \$)
Drought	Drought	2	–	–	800,000
Earthquake (seismic activity)	Earthquake (ground shaking)	30	115,621	1,029,121	33,484,852
	Average per event		3,854	34,304	1,116,161.7
Epidemic	Viral infectious diseases	2	3	10,001	–
	Average per event		1.5	5,000.5	–
Extreme temperature	Cold wave	2	–	–	–
	Extreme winter conditions	1	9	–	–
	Heat wave	3	20,105	–	4,400,000
	Average per event		6,701.7	–	1,466,666.7
Flood	Unspecified	15	492	1,485,020	2,930,000
	Average per event		32.8	99,001.3	195,333.3
	Flash flood	5	373	43,330	8,147,000
	Average per event		74.6	8,666	1,629,400
	General flood	15	182	1,336,962	11,718,600
	Average per event		12.1	89,130.8	781,240
Mass movement wet	Avalanche	1	1	–	5,510
	Landslide	13	2,584	19,596	1,353,700
	Average per event		198.8	1,507.4	104,130.8
Storm	Unspecified	11	188	6,000	2,328,700
	Average per event		17.1	545.5	211,700
	Extratropical cyclone (winter storm)	1	3	–	–
	Local storm	5	44	124	1,048,000
	Average per event		8.8	24.8	209,600
	Tropical cyclone	1	35	200	3,200
Volcano	Volcanic eruption	5	735	21,024	3,100
Wildfire	Forest fire	7	21	320	1,700,000
	Average per event		3	45.7	242,857.1

Source: EM-DAT (2011)

different characteristics depending on types of landslide. Generally, fast landslides are the result of heavy rainfall, while slow ones are caused by rains of medium intensity. For Italy, climate change would represent a general increase of fast landslides and decrease of slow ones (Bigano and Pauli 2007).

2.3 Impacts of Floods and Landslides on Socio-Economic Systems

Although floods are natural events in ecological and agricultural systems, they may cause considerable damages, including mainly loss of people lives. Serious damages are also due to the impact on houses and industrial structures of debris

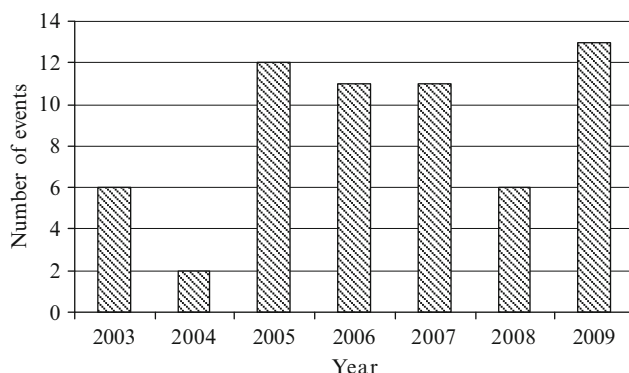


Fig. 2.1 Number of landslides in Europe from 2003 to 2009 (from Spizzichino et al. 2010)

or voluminous material violently transported by water, and to diseases spread by contamination of drinking waters. The latter may become extremely dangerous especially if toxic substances accumulated in sediments are released during floods in concentrations larger than those acceptable for human risk (Munich Re 1997). Violent floods can seriously affect agriculture through crop destruction, loss of livestock, and enhanced surface soil erosion in cultivated areas. Disruptions in transport and damage to civil infrastructure, as well as tourist and recreational areas, may hamper relief helpers and rapid return to normal conditions.

Landslides are more localized phenomena and hit smaller areas, thus causing less monetary damages than floods. For the 2003–2009 period, 61 landslides were recorded in the whole Europe (Fig. 2.1).

In the last 80 years, 5,400 floods and 11,000 landslides occurred in Italy (ISPRA 2011c). Because of the high impact of these events on landscape and human health, the Italian Ministry of Environment decided in 1997 to acquire a complete and homogeneous knowledge on landslides distribution throughout Italy, by funding a 4.1 million € IFFI Project (*Inventario dei Fenomeni Franosi in Italia* – “Italian Landslide Inventory”). In 2004, the Agency for the Protection of the Environment (APAT), now ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale), conferred 650,000 € to complete the databank. Up to December 31, 2007, the inventory had surveyed 482,272 landslides, covering an area of 20,500 km² (about 6.8% of Italy). The region where most landslides occurred is Lombardy, with 132,533 landslides (Fig. 2.2).

Landslides are complex dynamic phenomena encompassing different types of mass movements, based on which ISPRA classified the landslides events occurred so far in Italy: (1) 32.5% of total landslides were caused by rotational/translational movements, (2) 15.3% by slow mass flows, (3) 14.6% by rapid debris flow. In the IFFI Project, the relationship between landslides and slope steepness has been also analysed and it was found that slopes instability does not increase with slope angle, while only a specific range of slopes was statistically responsible for most landslides. The instability of a slope is often due to the interaction of several natural

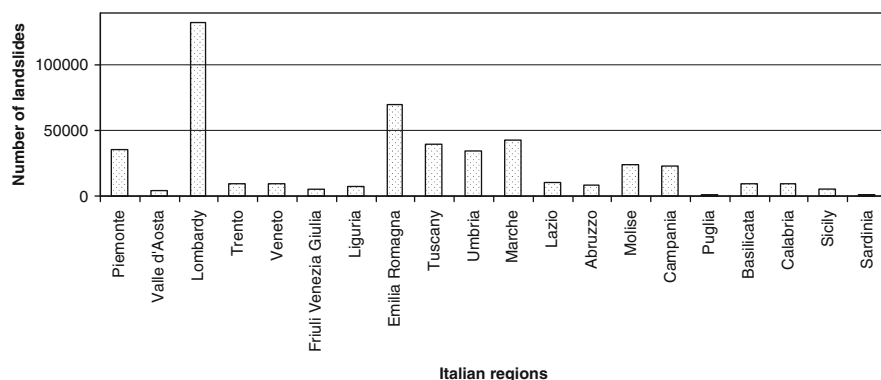


Fig. 2.2 Number of landslides per Italian region (elaboration from ISPRA 2008)

and/or anthropogenic causes. However, intense and short, as well as prolonged rainfall events are the most important factors for triggering slope instability phenomena (ISPRA 2008).

The socio-economic effects of landslides are similar to those of floods on agriculture, in terms of crop and livestock losses, and land subtracted from productivity, although the overall ecosystem disruption results smaller than for floods (Bigano and Pauli 2007).

According to APAT, Italy has funded 447.36 million € until 2006 to fight the flood risk and 667.88 million € to prevent landslides (Bigano and Pauli 2007), while it is documented (ISPRA 2011c) that Italy invested 2.81 billion of € to study and remediate landslides from 1990 to 2010 (Fig. 2.3).

2.4 Desertification and Climate Change

The UNCCD declares the desertification is a process of “degradation of arid, semi-arid and dry sub-humid soil environments resulted from several factors, including climatic variations and human activities” (UNCCD 1994).

The IPCC defines desertification as “the reduction or disappearance of biological or economic productivity and complexity of non-irrigated and irrigated croplands, pastures, forests or woodlands, which result from land uses or other phenomena originated from human activities and modes of settlement, including soil erosion by wind and water, the deterioration of physical, chemical, biological or economic properties of soil, and long-term disappearance of natural vegetation” (IPCC 2007a).

Desertification is thus constantly presented as a phenomenon due to both natural (climate and water cycle) and anthropogenic factors, which have an impact on how soil interacts with water cycle. In the IPCC Third Assessment Report (TAR), the role of unsustainable agricultural practices such as uncontrolled grazing and

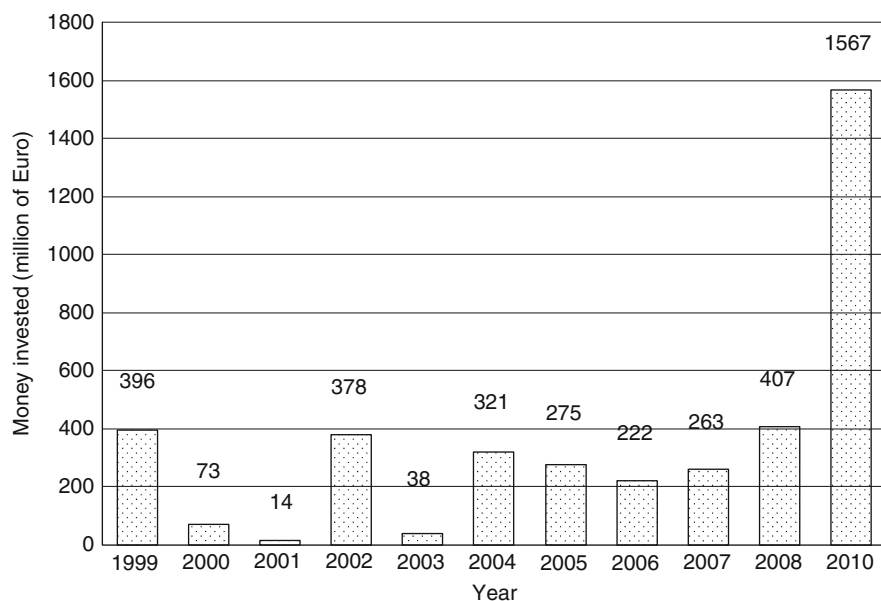


Fig. 2.3 Yearly investments (million euro) in Italy to remediate landslides risk and damage (ISPRA 2011c). The allocated resources allowed a total of 3,642 operations, though only 1,798 are completed and 698 are still ongoing (Fig. 2.4)

deforestation is particularly highlighted (IPCC 2001). TAR also indicates that climate change and human activities are strongly related to land use, involving synergistic impacts on ecosystems and species in desert areas. In fact, desertification is closely linked to characteristic climatic conditions such as drought, dryness, erosion, and rainfall, whose variation inevitably changes the intensity of desertification processes (Gambarelli et al. 2007).

The United Nations Convention to Combat Desertification (UNCCD) identifies physical, chemical, and biological processes of soil degradation. Physical processes lead to loss of resources in terms of reduction of soil volume and surface (erosion, compaction, and consequent limitation in porosity and water infiltration). Chemical processes cause the degradation of soil chemical quality (contamination, salinization, leaching, acidification). Biological processes determine the alteration of biological resources and their features (loss of organic matter, fertility, erosion resistance, buffering, and biodiversity).

According to the Italian National Atlas, risk of desertification in Italy pertains to more than 20% of total surface and rise to more than 40% in southern Italy (Costantini et al. 2007). The Atlas identifies various systems that contribute to desertification such as water erosion, urbanization, salinization, and drought. Sensitive areas would amount to a total of 9.1%, especially in Sardinia, Sicily, Puglia, and Calabria, though important areas with vulnerable soils are also present in Campania, Tuscany, and Lazio (Gambarelli et al. 2007). Desertification in southern Europe has an impact on:

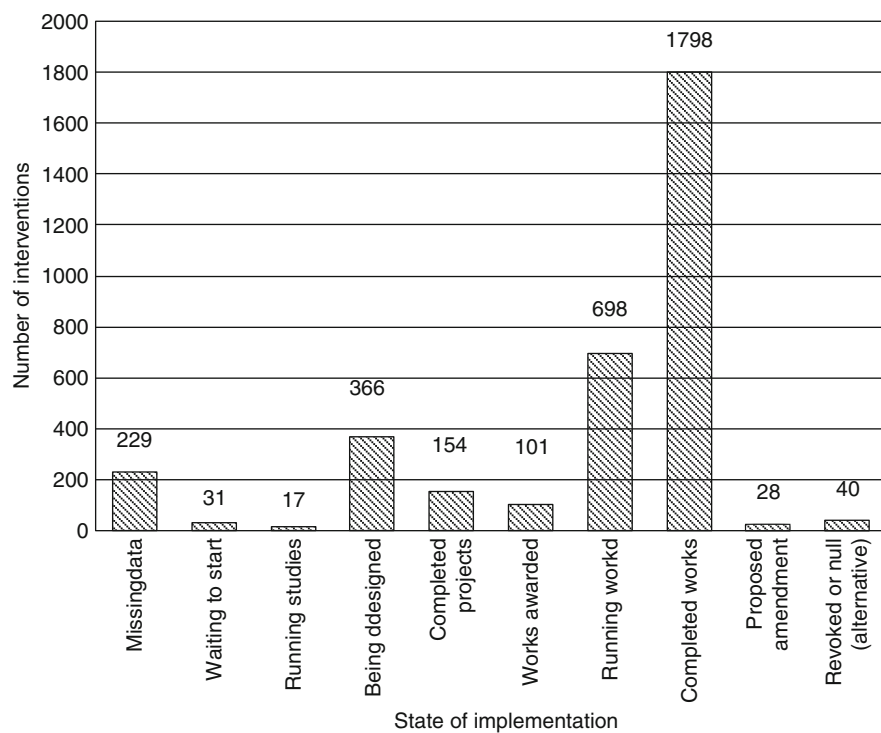


Fig. 2.4 Number of operations for landslides remediation based on their implementation state (ISPRA 2011c)

- Reduction in primary production and growth cycles of plants (Ogaya and Penuelas 2003).
- Reduction in turnover and availability of nutrients in soil (Sardans and Peñuelas 2005).
- Changes in phenology and interactions among species (Maestre and Cortina 2004).
- Risk of fire. A longer dry season increases frequency and severity of fires (Pereira et al. 2005) and a reduced capacity for vegetation recolonization after fires encourages the growth of shrubs over trees.
- Soil erosion due to increased intensity of rainfall events (Giorgi et al. 2004; De Luís et al. 2003).

2.5 Impacts of Desertification on Socio-Economic Systems

Desertification is a cause of direct and indirect social and economic effects. The first approximation to assess the costs related to reduced productivity is the estimate of soil physical damages and loss of fertile soil. A United Nations source in 1992

reported that Turkey, Tunisia, and Morocco lost due to soil erosion 54,237, 18,000, and 2,200 ha of cultivated land each year, respectively (UNEP 1992). A more recent study (Matallo 2006), based on the use of the Universal Soil Loss Equation (USLE), estimated that the loss of fertile soil may reach tens of billions of tons per year.

The only available economic estimate of desertification costs is based on the division of world arid areas in irrigated agricultural areas and non-irrigated grazing land (Dregne and Chou 1992). This classification was based on UNESCO reports for each country and was applied to estimate the economic cost of desertification per hectare, depending on land types or productive activities. Loss of yearly productivity per hectare due to soil degradation was calculated to be about 7 US\$ for grazing lands, 38 US\$ for non-irrigated land, and 250 US\$ for irrigated land. This study shows that the worldwide annual cost of desertification in 1990 amounted to 42 billion US\$, whose distribution for irrigated land, non-irrigated land, and pasture accounted to 11, 8, and 23 billion US\$, respectively.

Other studies have measured economic losses caused by land degradation in terms of agricultural Gross Domestic Product (GDP). Many of these studies are based on models of general equilibrium mostly related to developing countries (Diao and Sarpong 2007; Young 1999; Bojo 1996; Jebuni et al. 1994; Drechsel and Glielie 1999; ISSER/DFID/World Bank 2005). The estimates generally indicate an annual loss of agricultural GDP ranging from 2 to 10%, with a median around 5%. However, among the various possible effects of land degradation, these figures account only for loss of agricultural productivity and the impact that a productivity decline may have on the rest of national economies.

2.6 European and Italian Legislation Related to Climate Reporting

Under the UNFCCC agreement, all parties must report on the steps undertaken to implement the Climate Convention (Articles 4.1 and 12). This report is provided in a national communication that usually contains information on national approaches, such as vulnerability assessment, financial resources and transfer of technology, education and training. Additionally, information is made available on domestic policies and measures to limit or reduce GHG emissions and enhance their removal by sink mechanisms. The parties of Annex I were requested to submit the Fifth national communication to the secretariat by 1st January, 2010. Italy has already presented this report to the UNFCCC (MATTM 2009). At the end of March 2011, the Italian National Communication was subjected to a review process by experts of the UNFCCC and the review report will be soon available.²

²The review report for the Fifth National Communication will be available in: http://unfccc.int/national_reports/annex_i_natcom/idr_reports/items/2711.php

Parties have also submitted to secretariat the National GHG inventories of sources and sinks of anthropogenic GHG emissions, which are not controlled by the Montreal Protocol. Annual inventory submissions for Annex I Parties, consisting of *National Inventory Report*³ (NIR) and *Common Reporting Format*⁴ (CRF), are followed by an annual review of GHG inventories finalized with a report.⁵ In line with the reporting guidelines for national inventories from the IPCC, every year (x), parties shall report their anthropogenic GHG emissions by sources and sinks for the year $x-2$ (IPCC 1997, 2000, 2003). Detail information on methodologies and emission factors, emission time series, and uncertainties from Italian GHG emission inventory are reported in the NIR report (ISPRA 2011a). Since 2010, reports contain supplementary information as required by Article 7.1 of the Kyoto Protocol, such as those on Articles 3.3 and 3.4, for account of Kyoto units, changes in National Registry, and information on minimization of adverse impacts, in accordance with Article 3.14 of Kyoto Protocol.⁶

At European level, the legal basis for compilation of EC inventory is the 280/2004/EC (11/02/2004) Decision of the European Parliament and Council, concerning the mechanism for monitoring GHG emissions in EU and implementing the Kyoto Protocol. This decision established a mechanism to monitor, in each Member States, all anthropogenic GHG emissions (including their removal by sink mechanisms). Moreover, it requires the evaluation of progress made to ensure compliance with the EU commitments on emissions and their removal, the implementation of UNFCCC and Kyoto Protocol, and the guarantee that information reported by the European Commission to the UNFCCC Secretariat is complete, accurate, consistent, transparent, and comparable.

The Kyoto Protocol (Article 5.1) requires that each party included in Annex I establishes a *National system* for the estimation of sources and sinks of GHG anthropogenic emissions no later than 1 year prior to the start of the first commitment period. In Italy, the *National System* was set up by the Legislative Decree no. 51 of March 2008, designating ISPRA,⁷ formerly APAT,⁸ as the entity responsible to design, manage, and archive data of the *National System for the Italian Greenhouse Gas Inventory*, and to collect data and implement a program for monitoring

³ A comprehensive description of the methodologies used in compiling the inventory, the data sources, the institutional structures and quality assurance and control procedures.

⁴ A series of standardized data tables containing mainly numerical information and submitted electronically.

⁵ The last review report from Italy is available at the UNFCCC web site: http://unfccc.int/national_reports/annex_i_ghg_inventories/inventory_review_reports/items/5687.php

⁶ On 1st June 2002, Italy ratified the Kyoto Protocol with the law n.120 of 01/06/2002. The ratification law also prescribed the preparation of a National Action Plan to reduce greenhouse gas emissions, adopted by the Interministerial Committee for Economic Planning (CIPE) on 19th December 2002. The Kyoto Protocol entered into force on 16 February 2005.

⁷ ISPRA, Istituto Superiore per la Protezione e la Ricerca Ambientale.

⁸ APAT, Agenzia per la Protezione dell'Ambiente e per i Servizi Tecnici.

and quality assurance of data. The Italian *National System*, currently in place, is fully described in the document “*National Greenhouse Gas Inventory System in Italy*” (ISPRA 2011b). The “*National Registry for Carbon sinks*”, set up by a Ministerial Decree on April 1, 2008, is part of the Italian *National System* and includes information on lands units subjected to Article 3.3, activities listed under Article 3.4, and related carbon stock changes. The “*National Registry for Carbon sinks*” is the instrument to estimate the GHG emissions by sources and removal sinks for forest land and related land-use changes, and account for their net removal, thereby enabling the Italian Registry to deliver the relevant amount of removal units (RMUs).

2.7 Italian Greenhouse Gas Emissions

Emission estimates comprise ten GHG: carbon dioxide, methane, nitrous oxide, HFCs, PFCs, sulfur hexafluoride, nitrogen oxides, carbon monoxide, non-methane volatile organic compounds, sulfur dioxide. The first six GHGs directly contribute to climate change owing to their positive irradiative forcing effect. The national Kyoto target is a reduction in the period 2008–2012 of 6.5% of GHG, as compared to the 1990 base year level. In spite of it, total GHG emissions, in CO₂ equivalent, decreased by 5.4% between 1990 and 2009 (from 519 to 491 millions of CO₂ equivalent tons), excluding emissions and removals of carbon dioxide (CO₂) involved with LULUCF. Emission estimations include the following sources: energy, industrial processes, solvent and other product use, agriculture, LULUCF, and wastes. The most important GHG, CO₂, which accounted for 85% of total emissions in CO₂ equivalent in 2009, showed a decrease by 4.3% between 1990 and 2009. CH₄ and N₂O emissions were equal to 7.6 and 5.7%, respectively, of the total CO₂ equivalent GHG emissions in 2009. Both gases showed a decrease from 1990 to 2009, equal to 14.3 and 25.3%, respectively. Other GHG, HFCs, PFCs, and sulfur hexafluoride (SF₆) ranged from 0.04 to 1.7% of total emissions (ISPRA 2011a). The major contribution to the national GHG emissions is the energy sector (82.8%), followed by the agriculture (7.0%), and the industrial processes (6.1%) sectors (Table 2.2).

According to guidelines of IPCC national inventory for the agriculture sector, methane (CH₄) and nitrous oxide (N₂O) emissions should be estimated and reported. Enteric fermentation (4A), manure management (4B), rice cultivation (4C), agricultural soils (4D), and field burning of agriculture residues (4F) are included in this sector. In 2009, agricultural activity has been the dominant national source for CH₄ (41%) and N₂O (69%) emissions, and have decreased by 11.4 and 17.9%, respectively, in respect to 1990.

While the agricultural sector is responsible for 34.48 Mt of CO₂ equivalent, the GHG trend from 1990 to 2009 shows a decrease of 15.1% (Fig. 2.5). This was mostly due to a decrease of CH₄ emissions from enteric fermentation (−11.5%), and to a decrease of N₂O from agricultural soils (−20.6%), which account for 31 and 45% of total agricultural emissions, respectively (ISPRA 2011a). In particular,

Table 2.2 Greenhouse gas (Gigagrams, Gg) source and sink categories for Italy in 2009

GHG source and sink categories	2009 CO ₂ -equivalent (Gg)
Energy	406,743
Industrial processes	29,940
Solvent and other product use	1,862
Agriculture	34,481
Land use, land-use change and forestry ^a	−94,671
Waste	18,094
Total (including LULUCF removal) ^a	396,449
Total (excluding LULUCF)	491,120

Source: ISPRA (2011a)

^aIncludes net CO₂, CH₄, and N₂O

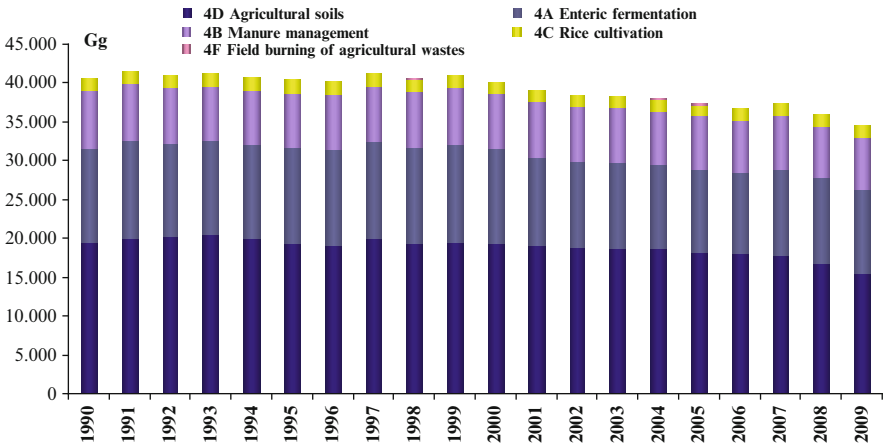


Fig. 2.5 Total GHG emissions from 1990 to 2009 for the agriculture sector (Gg CO₂ eq) (elaboration from ISPRA 2011a)

estimations from agricultural soils as source include (1) direct N₂O emissions from nitrogen fertilizers use, sewage sludge application, soil application of manure, N-fixing crops, incorporation of crop residues, histosols and pasture and (2) indirect N₂O emissions from atmospheric deposition and nitrogen leaching and run-off (ISPRA 2011a). Main drivers are the reduction in the number of animals, use of synthetic N-fertilizers, and agricultural production. Between 1990 and 2009, market interventions (I Pillar) due to Common Agricultural Policy (CAP), such as the milk quota, influenced reduction in number of dairy cattle (29%). Between 1990 and 2009, the use of N-fertilizers has been reduced by 37%, while the reduction was by 17 and 25% in the years 2007/2008 and 2008/2009, respectively. In addition, after the CAP Health Check reform, also Rural Development Plans 2007–2013 (II Pillar) will be likely to contribute to emission reduction by reducing nitrogen surplus (Córdoba et al. 2010).

The LULUCF sector includes estimations of CO₂ removal and emission of CO₂, CH₄, and N₂O for the following categories: *forest land, cropland, grassland,*

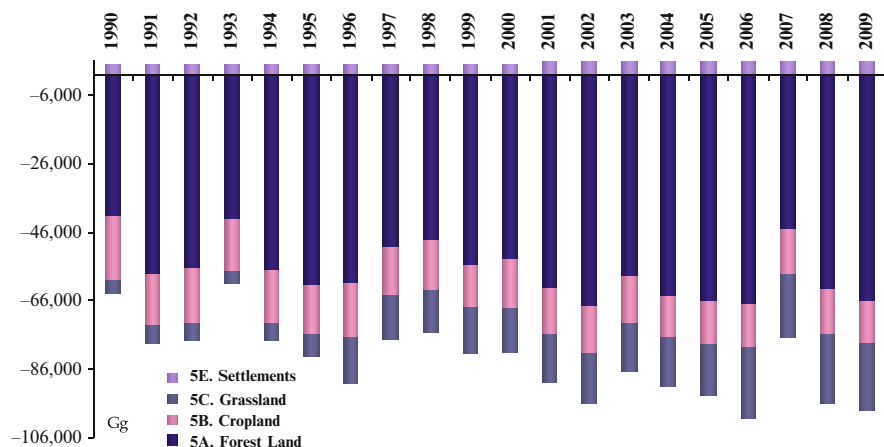


Fig. 2.6 Total emissions and removals from the LULUCF sector from 1990 to 2009 (Gg CO₂ eq) (elaboration from ISPRA 2011a)

wetlands, and settlements. Emissions and removals are estimated for each category, and each further subcategories “land remaining land” and “land converting to land”. The sector was responsible in 2009 for removal of 94.7 Mt of CO₂ from atmosphere (Fig. 2.6). From 1990 to 2009, total removal as CO₂ equivalent increased by 53.2%, CO₂ accounting for more than 99% of total emissions and removals of the sector (ISPRA 2011a).

Forest land removals accounted for 65% of total CO₂ LULUCF emissions and removals in 2009. In particular, the living biomass removals represented 50%, while removals from dead organic matter and soils stood for 8 and 42%, respectively, of total forest land CO₂ removed in 2009. The key driver for such rise was the increase in CO₂ removals from forest land remaining *forest land*.

Cropland removals were 12.1% of total CO₂ LULUCF emissions and removals. In particular, the living biomass removals represented 97%, while the emissions and removals from soils were up to 3% of total cropland CO₂ emissions and removals.

Between 1990 and 2009, mean *Grassland* emissions reached 13.6% of absolute CO₂ LULUCF emissions and removals. Living biomass emissions represented 7%, while removals from dead organic matter pool reached 3% and those from soils were up to 91% of absolute total grassland CO₂ emissions and removals (ISPRA 2011a).

2.8 Italian Research Supporting Climate Reporting

Two main lines are significant to improve estimations of GHG emissions from agriculture. A first line is related to collection of information on methods of agricultural production. For instance, agricultural statistics related to housing and storage facilities used by farmers and modality of land spreading are relevant

information required by the inventory. Currently, the National Institute of Statistics (ISTAT), with the support of ISPRA, incorporated these specific queries in the 6th Agricultural Census of 2010. Therefore, in the future, information on animal production methods will be obtained. A second line encompasses the collection and incorporation of country specific parameters as a function of national research studies. The GHG inventory already uses different Italian emission factors (i.e., rice cultivation emission factors). However, information on the national emission factor for N₂O emissions for agricultural soils is still needed.

In accordance with the COP/MOP Decisions, the IPCC Good Practice Guidance on LULUCF, and every relevant IPCC guidelines, the *National Registry for Carbon sinks* is the instrument to estimate GHG sources and sinks in forest land and related land-use changes. In 2009, a technical group, formed by experts from different institutions (ISPRA; Ministry of the Environment, Land and Sea; Ministry of Agriculture, Food and Forest Policies), set up the methodological plan for the necessary activities to implement the registry, and defined the relative funds. Some of these activities which should be completed by 2010 are expected to supply useful data to update and improve present estimations. Activities planned in the framework of the *National Registry for Forest Carbon Sinks* should also provide data to improve estimate of carbon sequestration due to Afforestation/Reforestation activities (with a special focus on soil organic matter content), and should allow to refine the estimates for forest land category. For the 2011 report submission, data and methodologies used for the inventory under the Convention were employed to estimate emissions and removals for activities related to Articles 3.3 and 3.4.

In recent years, different national research studies have focused on carbon sequestration in agricultural soils, highlighting the relationship between agricultural management practices and soil organic matter content. In fact, soil organic matter content represents a critical issue for Italy due to the small amount of soil surveys conducted since 1990, and their extreme fragmentation. Concerning forest land, results of the third phase of the National Forest Inventory (INFC) based on measurements of soil organic matter content will enable a more accurate analysis of the relationship between biomass carbon and carbon content in litter and soil, thus providing an improvement of national and regional estimates.

However, the issue is still open for soil organic matter content related to other land uses, particularly for cropland and grassland. Recent studies have reported that soil organic carbon was reduced in many areas, while an increase in atmospheric CO₂ has been concomitantly detected. This apparently shows that past changes in land use history and management practices were the main drivers for carbon emissions from soil rather than high temperatures and rainfall changes resulting from climate change (Fantappiè et al. 2010). Further investigations are needed to develop a solid methodology to assess carbon stock changes in soil pool and provide a robust database over the national territory with precise records of soil organic matter content vis-à-vis of land use and management practices.

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Chapter 3

Field Plots and Crop Yields Under Innovative Methods of Carbon Sequestration in Soil

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Abstract This chapter reviews the issues related to the responses of crops and soil fertility to management strategies aimed to conserve soil carbon, especially for Mediterranean-Temperate conditions. It reports the main results from field experiments conducted in three different Italian sites in order to compare traditional and innovative soil treatments for carbon sequestration. Field agronomic treatments included traditional and minimum tillage, green manuring, two rates of mature compost application, and spreading of water-soluble Fe–porphyrin. Their effects were tested in different sites representing distinct pedo-climatic conditions.

3.1 Introduction

Cultivation of soils plays a crucial role in transferring carbon from terrestrial to atmospheric pools. A source of carbon comes from land use change from natural to agricultural conditions that implies the reduction of woody biomass and amount of residue returned to soil, as well as the increase of soil organic matter (SOM) mineralization. On the other hand, decreasing CO₂ emissions from soil to atmosphere means to return the exceeding atmospheric C into the terrestrial pool, and sequestering carbon in soil turns soils from sources to sinks of carbon (Paustian et al. 2000).

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SOM is recognized as the major factor controlling the capacity of soils to deliver agricultural and environmental services (Manlay et al. 2007). Therefore, it is evident that enlarging soil C stock has not only a pivotal function in reducing atmospheric CO₂, but has several other positive advantages for agricultural systems, in terms of plant growth support, nutrients cycling, and water cycle regulation (Bauer and Black 1994; Mondini and Sequi 2008). SOM is indeed crucial for maintaining soil aggregation and aeration, hydraulic conductivity and water availability, cation exchange and buffer capacity, and supply of mineralizable nutrients (Khan et al. 2007).

Soil C content is the balance between C inputs and C outputs (Paustian et al. 1997; Lal 2004; Rees et al. 2005). Management practices aimed at enhancing soil C content can be addressed either to decrease C losses or to increase C supply.

In soils of southern Europe, SOM content is usually very small due to common synergy between natural and human-induced factors that induce SOM degradation (Zdruli et al. 2004). Southern Europe is mainly influenced by Mediterranean climatic conditions (i.e., cool humid winters and warm dry summers), which do not favor SOM accumulation. In hyperhumid regions, SOM accumulation is due to anaerobic conditions that reduce microbial activity (Satrio et al. 2009). Conversely, in temperate regions, SOM content generally increases with increasing rainfall, because larger soil moisture results in greater plant biomass that, in turn, provides more carbon residue and, thus, more potential food for soil biota. Moreover, high temperature and water availability accelerates SOM decomposition and finally result in low SOM content in such temperate conditions (Post et al. 1982).

In respect to human-induced factors, it is well known that land use change from natural to cultivated is one of the major causes of SOM degradation (Bruce et al. 1999; Guo and Gifford 2002; Celik 2005). In the Mediterranean basin, human pressure has been particularly high since at least 3,000 years. The needs of Mediterranean societies were satisfied by wide deforestation to allow intensive land management and exploitative agriculture (Blaikie and Brookfield 1987).

Soil tillage is the cultivation technique that exerts the major impact on SOM degradation due to inherent alteration of oxidative conditions, soil structure, and biodiversity (Fagnano and Quaglietta Chiarandà 2008). Conservation tillage techniques, minimizing soil inversion and soil structure disruption, have shown to increase SOM (Holland 2004). However, more complex strategies of conservation agriculture with integration of tillage and other practices are necessary to counteract soil degradation. As shown by long-term field experiments, the combined use of different techniques, such as long-time rotations (including permanent meadows which reduce tillage frequency), manuring, conservation tillage, and crop residue return to soils, led to a significant increase in SOM content (Reeves 1997).

Agronomic techniques employing secondary crops as cover crops, green manuring and live mulching, resulted particularly effective in limiting soil degradation (Wagger et al. 1998; Steenwerth and Belina 2008b). In addition, secondary crops strongly interact with the plant–soil nitrogen cycling and alter microbial activity in the rhizosphere (Steenwerth and Belina 2008a), thus also affecting SOM content in the long term. Similarly, soil mulching with crop residues proved to increase SOM content and soil fertility (Holland and Coleman 1987; Bot and Benites 2005).

Enhancing residues return to soils implies primary production growth, but also the increase of the nonharvested crop portions. Thus, the removal of crop residue for biofuels, devised as a possible plan for alternative energy production, creates a negative nutrient budget and can lead to soil degradation (Lal et al. 2004). Cropping systems and management practices which ensure large amounts of crop residue returned to soil are generally expected to cause a net build-up of SOC stock (Gregorich et al. 1996). Nevertheless, the addition of easily decomposable C compounds to soil may concomitantly induce an acceleration of mineralization processes by the priming effect and counterbalance the tendency in enriching the soil organic C pool (Kuzakov et al. 2000).

Carbon is supplied to soil also via animal manure and compost. Both these practices imply the recycle of biomasses (from animal livestock, food industry, garden and urban wastes) and reduction of chemical fertilizers additions. Besides the contribution for carbon sequestration, the use of organic fertilizers enables the reduction of other greenhouse gases, thus diminishing both the energy required for unit of available nutrient and emissions of N_2O after soil incorporation (Freibauer et al. 2004; Smith 2004; Alluvione et al. 2010).

Application of polymeric soil conditioners was applied in the past to attempt SOM stabilization (Wallace 1986) without much success. Recently, Piccolo and coworkers have introduced the concept of in situ photo-polymerization of SOM under the action of a biomimetic catalyst such as a synthetic Fe-porphyrin (Piccolo et al. 2005; Smejkalova and Piccolo 2005; Smejkalova et al. 2006). This innovative practice should enhance the molecular mass of humic molecules and, thus, their biochemical recalcitrance in soil (see Chaps. 1 and 4). The method has been tested in pot experiments (Piccolo et al. 2011) and microcosms (Gelsomino et al. 2010), and carbon sequestration in unplanted soils was successfully shown.

All agronomic practices which intend to promote soil C sequestration do strongly interfere with water balance and nutrient cycling in soil. Their impact on crop yield and SOM is potentially great, but actual effects are dependent on specific cropping systems and environmental conditions. It is therefore essential to study their efficacy in different conditions, since the application of practices developed in other climates can lead to unexpected results. For example, the beneficial effects of reduced tillage and cover crops are not obvious in Mediterranean climate zones, whereby a potential reduction of soil water content at crucial stages of crop growth, such as germination and grain filling, may severely affect crop production.

3.2 Field Research Activity Within the MESCOSAGR Project

3.2.1 *Site Description*

The agronomic effects of different soil treatments aimed at promoting soil C sequestration were tested in different Italian pedo-climatic conditions, all belonging

to mesothermal climates (hot temperate climates suitable for a good agriculture), according to the Strahler and Strahler classification (1984).

Field experiments were set in three different sites: Site 1 – located in the Po River Valley, in northern Italy (type F climate: hot temperate climate without dry season, hereafter “Temperate”); Sites 2 and 3 – located in the coastal plains of southern Italy (type S climate: hot temperate climate with dry summer, hereafter “Mediterranean”). Site locations are the following:

1. Torino (Tetto Frati experimental station, University of Torino, 44°53'N, 7°41'E, 232 m a.s.l.), here identified as TO.
2. Napoli (Torre Lama experimental station, University of Napoli, 40°37'N, 14°58'E, 30 m a.s.l.), here identified as NA.
3. Battipaglia (Experimental station, University of Basilicata, 40°35'N', 14°59'E, 65 m a.s.l.), here identified as BA.

The experimental sites were chosen as examples of typical conditions of agriculture in southern Europe that is often poorly represented in scientific literature. Torino (TO) and Napoli (NA), with different climates and soils, were selected to evaluate the same treatments in different pedo-climatic conditions. Battipaglia (BA), similar to NA as for pedo-climatic conditions, was added to evaluate the same treatments on a different crop. Field trials were conducted for 3 years, i.e., from 2006 to 2008 in TO and NA, and from 2007 to 2009 in BA.

3.2.2 Meteorological Conditions

From the analysis of the main meteorological parameters (Tables 3.1 and 3.2), it is evident that the main differences among sites are during the winter period. In TO,

Table 3.1 Main meteorological parameters of the three sites

Year	Temperature (°C)			Rainfall (mm)		ET ₀ (mm)	
	Annual average	Daily maximum	Daily minimum	Annual total	Daily maximum	Annual total	Daily maximum
Torino							
2006	12.7	35.2 (Jul 22)	−8.9 (Jan 15)	605	122 (Sep 14)	1,102	7.0 (Jun 23)
2007	12.9	34.1 (Jul 19)	−8.4 (Dec 18)	513	44 (May 4)	1,138	7.1 (Jul 16)
2008	12.6	34.7 (Aug 5)	−11.4 (Dec 28)	1,071	62 (Jun 8)	1,072	6.9 (Jun 27)
Napoli							
2006	16.7	38.0 (Aug 19)	−2.3 (Jan 26)	932	64 (Nov 12)	1,143	6.9 (Jun 28)
2007	17.2	42.1 (Aug 24)	−1.4 (Dec 15)	726	37 (Nov 14)	1,126	7.4 (Jul 24)
2008	17.1	35.6 (Sep 6)	+0.1 (Mar 16)	853	53 (Mar 6)	1,136	6.9 (Jun 17)
Battipaglia							
2007	17.6	42.5 (Aug 24)	+0.0 (Dec 16)	951	49 (Sep 27)	1,164	7.6 (Jul 24)
2008	17.9	37.0 (Jul 13)	−0.5 (Feb 17)	1,028	66 (Mar 6)	1,175	7.4 (May 27)
2009	18.0	39.0 (Aug 21)	+0.5 (Feb 21)	1,324	89 (Jan 3)	1,154	6.8 (Jul 25)

Table 3.2 Water balance (Rain – Reference Evapotranspiration) for the three sites

Year	Jan–Apr	May–Aug	Annual total
Torino			
2006	–93	–404	–497
2007	–199	–427	–626
2008	–58	–215	–371
Napoli			
2006	103	–446	–212
2007	–1	–524	–400
2008	137	–503	–284
Battipaglia			
2007	89	–512	–212
2008	147	–552	–148
2009	478	–516	170

temperatures are generally lower, with winter negative peaks around -9°C , while winter peaks in Mediterranean sites ranged from -2.3 to $+0.5^{\circ}\text{C}$. Annual rainfalls are smaller in TO, larger in NA, and much larger in BA. Reference evapotranspiration (Hargreaves and Samani 1985) is not different among the three sites, with total annual values ranging from 1,072 to 1,164 mm. In winter, water balance (annual rainfalls minus total evapotranspiration) is negative in TO and positive in the Mediterranean sites (Table 3.2), while it is negative in summer with a more severe drought in the Mediterranean sites (NA, BA). On a year basis water deficit is severe in TO (-498 mm on average), moderate in NA (-299 mm), and negligible in BA (-63 mm).

The differences among sites are noticeable from the monthly distribution of evapotranspiration and rainfalls. Summer rainfalls are abundant in the Temperate site, while the Mediterranean sites show more abundant winter rainfalls and severe summer droughts.

These differences are due to the origin of rainfalls. In Italy, temperate-humid storms during fall and winter mainly move from Atlantic Ocean. In northern Italy, storms encounter the Alps Mountains. Consequently, a wetter climate prevails on the windward side (French Provence) than on the leeward side as moisture is removed by orographic precipitation. Therefore, in the Western Po Valley (where the Torino site is located) precipitations are little. On the contrary, in southern Italy storms encounter the Apennines Mountains which determine wetter climate in the windward side (Tirrenic coast) where the NA and BA sites are located.

During summer, convective precipitations occur in the Po valley as the warm-humid air masses rise vertically from the very wet Po Plain and encounter colder air, thus condensing and determining intense rainfalls. In southern Italy, drought is more severe in summers. Thus warm air masses are much dryer, determining little summer convective rainfalls.

Table 3.3 Soil properties in the three sites

Property	Soil depth (cm)								
	Torino			Napoli			Battipaglia		
	0–15	15–30	30–60	0–15	15–30	30–60	0–15	15–30	30–60
Sand (%)	62.4	63.1	50.8	47.0	46.0	41.5	11.8	13.0	12.8
Silt (%)	30.2	30.0	41.1	20.1	24.5	28.5	57.3	54.3	46.4
Clay (%)	7.4	6.9	8.1	32.9	29.5	30.0	31.0	32.7	40.8
Texture class	SaL	SaL	L	SiCL	SiCL	CL	SiCL	SiCL	SiC
pH	8.1	8.0	8.1	7.4	7.4	7.5	8.2	8.1	7.9
Total carbonate (%)	2.8	2.0	4.3	–	–	–	–	–	–
Organic matter (%)	1.78	1.70	1.22	1.24	1.24	1.11	1.10	1.18	0.68
Organic C (%)	1.04	0.99	0.71	0.72	0.72	0.64	0.63	0.68	0.39
Nitrogen (%)	0.104	0.104	0.079	0.093	0.090	0.062	0.081	0.068	0.041
C/N ratio	10.0	9.5	9.0	7.74	8.00	10.40	7.75	10.00	9.51
K ₂ O (ppm)	75	61	45	440	420	432	392	401	380
P ₂ O ₅ (ppm)	35	38	13	31	32	25	49	31	24

Sa sandy, L loam, Si silty, C clay

3.2.3 Soil Properties

Soil properties are different among experimental sites (Table 3.3), with soil texture the most distinct. In top layers, soil texture was sandy loam for TO with a low content of clay (7–8%), while it was silty clay loam in the other two experimental sites with a clay content of 30–33% at NAP and of 31–40% at BAS.

SOM was larger in TO (1.7–1.8%) than in both NA (1.2%) and BAS (1.1–1.2%). Total N was greater and exchangeable K₂O smaller in TO than for the other two sites. Soil classification (USDA soil taxonomy) was (1) Typic ustifluent in TO, (2) Vertic Haploxeralf in NA, (3) Pachic Haploxeroll in BA.

3.2.4 Experimental Set-Up and Soil Climatic Conditions for Maize Trials

Maize (TO and NA) and sorghum (BA) were grown in the field sites because their large N demand is liable to better reveal the treatment effects.

Soil treatments at the TO and NA sites were the following:

1. TRA = traditional soil tillage (moldboard plowing, 30 cm deep), mineral fertilization with urea at the rate of 130 kg ha⁻¹ of N.
2. MIN = minimum tillage (rotary hoeing, 10 cm deep), mineral fertilization with urea at the rate of 130 kg ha⁻¹ of N.
3. GMAN = green manure with hairy vetch (*Vicia villosa* L.), moldboard plowing, 30 cm deep.

4. COM1 = low rate of compost (equivalent to 130 kg ha⁻¹ of N), moldboard plowing, 30 cm deep.
5. COM2 = high rate of compost (equivalent to 260 kg ha⁻¹ of N), moldboard plowing, 30 cm deep.
6. CONT = no N fertilization, moldboard plowing, 30 cm deep.

Soil treatments at the BA site were the following:

1. TRA = traditional soil tillage (moldboard plowing, 40 cm deep), mineral fertilization with urea at the rate of 130 kg ha⁻¹ of N.
2. COM1 = low rate of compost (equivalent to 130 kg ha⁻¹ of N), moldboard plowing, 40 cm deep.
3. COM2 = high rate of compost (equivalent to 260 kg ha⁻¹ of N), moldboard plowing, 40 cm deep.
4. CONT = no N fertilization, moldboard plowing, 40 cm deep.

A completely randomized design with four replications was adopted for the TO site. Field plots ($6 \times 8 = 48 \text{ m}^2$) were 24 (6 treatments $\times$ 4 replications).

A randomized complete block design with four replications was adopted for the NA site. Field plots ($6 \times 5 = 30 \text{ m}^2$) were 24 (6 treatments $\times$ 4 replications).

A randomized complete block design with four replications was adopted for the BA site. Field plots ($5 \times 8 = 40 \text{ m}^2$) were 16 (4 treatments $\times$ 4 replications).

Fertilization was carried out in spring just before moldboard plowing. Chemical composition of the compost used in all the three sites is reported in Table 3.4. Vetch crop (*Vicia villosa* L., cv. Haymaker plus) for green manure was sown at TO and NA sites in fall with a seed rate of 280 kg ha⁻¹ and shredded at the same time of fertilizers addition. Phosphorus and K fertilization ($\text{P}_2\text{O}_5 = 100 \text{ kg ha}^{-1}$; $\text{K}_2\text{O} = 200 \text{ kg ha}^{-1}$) was the same on all plots.

Maize crop (*Zea mays* L., hybrid PR34N43, FAO 500, Pioneer Hi-Bred) was sown at TO and NA at late spring with a density of 7.4 seeds per m². Sorghum crop (*Sorghum bicolor* Moench $\times$ *S. sudanense* (Piper) Stapf. cv. BMR333, Società Italiana Sementi) was sown at BA in late spring with a density of 20 plants per m². Irrigation was made with a traveling-gun sprinkler irrigation system in TOR and with a drip irrigation tape system in NAP and BAS. Irrigation volumes were

Table 3.4 Chemical composition of the compost added to plots each year

	2006	2007	2008
Dry matter (% f.m.)	56.9	61.0	61.6
Organic matter (% d.m.)	52.1	51.1	45.4
Organic C (% d.m.)	33.0	25.6	22.7
Hemicellulose (% d.m.) ^a	12.4	10.7	9.4
Cellulose (% d.m.) ^a	10.6	12.0	8.4
Lignin (% d.m.) ^a	12.8	11.2	12.7
Ashes (% d.m.)	33.7	39.1	47.9
Total N (% d.m.)	1.9	2.1	2.3
C/N ratio	15.9	12.2	9.8

^aFrom Van Soest et al. (1991)

decided on the basis of crop evapotranspiration calculated by multiplying reference evapotranspiration (Hargreaves and Samani 1985) by crop coefficients (Allen et al. 1998) in TO and NA. At BA, irrigation water was applied every 3–4 days with the aim to maintain soil moisture around field capacity by restoring at each irrigation the amount of lost soil water calculated through TDR readings from probes buried up to 210 cm of depth.

Total amount of water (rainfalls + irrigation) corresponded to 70, 59, and 71% of ETP, respectively, in the 3 years in TO, 76, 77, and 88% of ETP in NA and 103, 97, and 98% of ETP in BA (Table 3.5). A detail of all agronomic techniques is reported in Table 3.6.

For agronomic analyses, soil samples were collected before sowing and after harvest from all fields and for all years in three soil layers (0–15, 15–30, 30–60 cm) and the N-NH_4 and N-NO_3 content was determined.

For Torino, soil mineral N was extracted by shaking 100 g of moist soil with 300 ml of 1 M KCl solution for 1 h. Subsequently, the samples were filtered through a Whatman no. 1 paper, and then the extracts were frozen until analysis. $\text{NO}_3^- - \text{N}$ and $\text{NH}_4^+ - \text{N}$ concentrations were determined by colorimetry with a continuous flow analyzer (Evolution II, Alliance Analytical Inc., Menlo Park, CA).

For Napoli, soil mineral N was extracted with Hach standard kit reagents (Hach DR 2000, Hach Company, Loveland, CO) and the extracts were analyzed by the spectrophotometer at 500 ($\text{NO}_3^- - \text{N}$) and 425 ($\text{NH}_4^+ - \text{N}$) nm. Mineralization of soil organic N was estimated by N uptake in nonfertilized control, plus soil mineral variation between crop sowing and harvest (Meisinger 1984; Bhogal et al. 1999; Fiorentino et al. 2009). Plant samples were collected at harvest and oven-dried at 70°C to constant weight. A sub-sample of dry biomass was analyzed for N content (Kjeldhal method at NA and elemental analyzer at TO and BA).

Analysis of variance was applied to determine differences among treatments in total biomass, N content, and N uptake. Data were analyzed for separated sites and treatments as main factor, while year was the repeated measure. When effects were significant, means were separated using a Sidak post hoc test.

Table 3.5 Water supply during cropping years in the three sites

Year	Rainfalls	Irrigation	Total water supply	ETP	Water supply/ETP ratio
Torino					
2006	309	160	469	667	0.70
2007	229	140	369	628	0.59
2008	432	40	462	654	0.71
Napoli					
2006	148	275	423	555	0.76
2007	5	345	350	453	0.77
2008	109	362	471	538	0.88
Battipaglia					
2007	26	512	538	522	1.03
2008	3	458	461	475	0.97
2009	71	362	433	442	0.98

Table 3.6 Schedule (day/month) of maize and sorghum agronomic operations at the three sites

Torino	Napoli	Battipaglia	Operations
15/3, 2006	11/11, 2005	–	Seedbed preparation and vetch sowing
26/10, 2006	3/11, 2006		
23/10, 2007	7/11, 2006		
17/5, 2006	4/5, 2006	–	Vetch sampling and chopping
17/5, 2007	10/5, 2007		
17/5, 2008	21/5, 2008		
18/5, 2006,	5/5, 2006	–	Vetch incorporation
4/5, 2007	12/5, 2007		
19/5, 2008	21/4, 2008		
18/5, 2006	4/11, 2005	27/4, 2007	Main tillage according to experimental protocol
4/6, 2007	8/11, 2006	30/4, 2008	
19/5, 2008	11/10, 2007	8/5, 2009	
18/5, 2006	17/5, 2006	24/5, 2007	Fertilization and seedbed preparation
4/6, 2007	24/5, 2007	10/6, 2008	
19/5, 2008	5/5, 2008	11/6, 2009	
19/5, 2006	22/5, 2006	31/5, 2007	Maize or sorghum sowing
4/6/5, 2007	8/6, 2007	14/6, 2008	
19/5, 2008	6/5, 2008	13/6/5, 2009	
8/6, 14, 2006	16/6, 20/7, 2006	24/6, 16/7, 2007	Weed control
6/6, 25, 2007	20/6, 23/7, 2007	27/6, 7/7, 2008	
19/6, 2008	18/6, 22/7, 2006	22/6, 6/7, 2009	
2006: 160 mm × 4	2006: 275 mm × 12	2007: 512 mm × 19	Irrigations
2007: 140 mm × 3	2007: 345 mm × 12	2008: 458 mm × 21	
2008: 40 mm × 1	2008: 362 mm × 11	2009: 362 mm × 22	
22/9, 2006	7/9, 2006	4/9, 2007	Harvest
10/10, 2007	9/9, 2007	9/9, 2008	
29/9, 2008	20/8, 2008	2/9, 2009	

3.2.5 Soil Amendment with Biomimetic Catalyst Under Wheat Trials

The action of the iron–porphyrin biomimetic catalyst, at the rate of 10 kg ha^{−1}, was tested at TO and NA in soils cropped with wheat (*Triticum aestivum* L., cv. Blasco). In 10 m² plots (three replications per site), a 1 m² soil surface was identified where iron–porphyrin was distributed before wheat emergence at the rate of 1 g m^{−2} (previously dissolved in 2 l of water). All plots were plowed at 30 cm and wheat was fertilized at the rate of 130 kg N ha^{−1} with ammonium nitrate. Phosphorus and K fertilization (P₂O₅ = 100 kg ha^{−1}; K₂O = 200 kg ha^{−1}) was the same on all plots. A detail of applied agronomic practices is reported in Table 3.7.

Plant samples were collected at harvest and oven dried (60°C) to measure yield and yield fractions. A sub-sample of dry biomass was analyzed (Kjeldhal method at NA and elemental analyzer at TO) to calculate N uptake by crops. At both sites a randomized complete block design with three replications was adopted. Field plots (2 × 5 = 10 m²) were 6 (2 treatments × 3 replications).

Table 3.7 Schedule (day/month) of wheat agronomic operations at the three sites

Torino	Napoli	Operations
14/3, 2006	4/11, 2005	P and K fertilization
26/10, 2006	8/11, 2006	
22/10, 2007	6/11, 2007	
16/3, 2006	4/11, 2005	Soil tillage
26/10, 2006	8/11, 2006	
22/10, 2007	18/10, 2007	
15/3, 2006	5/11, 2005	Seedbed preparation and wheat sowing
26/10, 2006	9/11, 2006	
23/10, 2007	6/11, 2007	
4/4, 2006	11/11, 2005	Fe–porphyrin treatments
15/11, 2006	1/12, 2006	
9/11, 2007	21/11, 2007	
19/4, 5/5, 2006	14/2, 2006	N fertilization
2/3, 29/3, 2007	15/2, 2007	
5/3, 24/4, 2008	22/2, 2008	
24/7, 2006	26/6, 2006	Harvest
3/7, 2007	28/7, 2007	
25/7, 2008	2/7, 2008	

3.3 Effects of Soil Tillage

Deep soil tillage is the crop management with most impact on soil properties (Fagnano and Quaglietta Chiarandà 2008). Soil tillage may have contrasting agronomic and environmental effects on soil structure and aeration of the tilled layer. The destruction of soil aggregates decreases macroporosity and infiltration rate, with enhanced risk of soil erosion and reduced oxygen diffusion rate. The consequent anaerobic conditions limit root growth and may increase denitrification and methanogenesis with enhanced greenhouse gas emissions (CH_4 and N_2O). Conversely, a macroporosity increase can be favorable in clayey soils (Pagliai et al. 2004). Increased aeration can stimulate SOM oxidation and nitrification (Reicosky et al. 1995), with positive effects on plant N uptake and growth, but negative effects on nitrate pollution of water table and CO_2 emissions from soils.

Literature reports contrasting findings on the impact of soil tillage on crop yield and environmental quality, depending on soil (texture, structure, SOM content) and weather (rainfall distribution and intensity) conditions, as well as cropping systems (crop, rotation, and management of crop residues) (Lal 1989; Barberi 2006). Moldboard plowing is considered the soil tillage method most harmful to SOM maintenance, because of deep soil layer disturbance and dilution of fresh organic matter (i.e., crop residues, organic fertilizers) in the soil profile (Pagliai et al. 1998).

Increasing soil disturbance passing from no-tillage (zero-tillage), to minimum tillage, to deep plowing has been shown to enhance potential SOM oxidation and microbial respiration (Morris et al. 2004). Long-term experiments (Tebrügge and During 1999) demonstrated that reduced tillage increased aggregate stability

and soil cover by crop residue, limiting surface sealing and erosion. Moreover, organic matter and nutrients increase in the top layer, while biological activities (i.e., earthworm) and water infiltration rates are enhanced. No tilled soils were also found to be more resistant to vehicle passage, thus reducing soil compaction.

In clayey soils, increase of SOM content following no-tillage was limited in the top layer (10 cm), while the negative impact in deeper layers (lower porosity and greater compaction) induces root growth reduction and maize yield losses (Mariotti et al. 1998). On the contrary, in sandy soils, reduced tillage increased soil porosity and crop yields of potato, wheat, burley, and oat (Ekeberg and Riley 1997).

In humid environments of Scotland, no-tillage caused yield losses due to lower permeability, waterlogging, and weed increase (Ball and Ritchie 1999). No-tillage determined a reduction of porosity and subsequent losses in wheat and maize yield in a silty soil (Cereti and Rossini 1995), while the porosity reduction did not affect root growth in deeper layers of sandy soils (Venezia et al. 1995).

Minimum tillage is believed to increase SOM content. However, it has been reported that its application can only determine a change of OM distribution in the soil profile, with larger content in the top layers and lower content in the deeper ones, without really altering its total content (Carone et al. 2000a; Triberti et al. 2000). Minimum tillage was found to reduce the yield of durum wheat and chickpea in some clayey soils of southern Italy (Mori et al. 2000). In general, reduced tillage may provide positive effects in dry environments, whereas it may bring very negative consequences on erosion and denitrification in regions with heavy rainy periods (i.e., in fall–winter period over Mediterranean clayey soils).

A lower impact of soil tillage is reported for spring–summer crops and in dry years (Marenghi et al. 2000), thus confirming that deep tillage is necessary in more rainy conditions and mostly in clayey soils (Carone et al. 2000b), while reduced tillage is more useful in dry conditions (Mori et al. 2000; Simanskaite et al. 2009).

3.3.1 Crop Yields from Soil Treatments Under Maize

Results obtained during the MESCOSAGR project indicate that reduced tillage did not influence maize growth. Biomass yield of maize (Table 3.8) from Torino and

Table 3.8 Biomass yield of maize crop (ton ha⁻¹ day month) in the three experimental sites

Site Treatments	Torino			Napoli			Battipaglia		
	2006	2007	2008	2006	2007	2008	2006	2007	2008
CONT	28.4	21.1	13.1	21.5	9.3	11.4	14.4	13.0	8.1
TRA	25.4	23.9	19.4	21.9	18.7	15.5	24.2	20.3	11.2
MIN	21.9	24.4	19.7	22.0	21.2	16.9	–	–	–
COM1	23.6	23.8	16.4	7.0	6.7	6.8	15.9	16.4	9.0
COM2	23.6	22.8	18.1	9.5	8.6	9.3	21.7	17.9	8.2
GMAN	26.6	24.4	19.7	21.5	17.1	12.5	–	–	–
Protected LSD (Sidak)	4.1			5.1			5.2		

Napoli field plots subjected to minimum tillage and moldboard plowing was not significantly different. Nevertheless, a positive correlation between yield variation (YV%) due to tillage method (MIN vs. TRA) and summer water deficit (WD) was found in Napoli site ($YV\% = -72.1 + 0.162 \text{ WD}$; $R^2 = 0.995$; $P \leq 0.05$).

This is in line with positive effects of reduced tillage practices on water availability and on plant growth found in drier conditions (Simanskaite et al. 2009; Marenghi et al. 2000; Mori et al. 2000).

3.4 Effects of Cover Crops, Mulching and Green Manure

Cover and catch crops, mulch, residue, and green manure provide many services to agroecosystems, from erosion and weed control to deposition and protection of organic matter (Jackson 2000) and promotion of genes for disease resistance (Kumar et al. 2004). Some of such services reduce yield of the main crop, especially in intercropping systems and in environments where water and/or nutrients availability are limited. Therefore appropriate management is required, and trade-offs between ecological benefits and production must be approached with solutions that are hardly transferable and need to rely on research conducted in relevant conditions.

Dead mulch, green manure, and crop residues left in the field provide OM to soil, while cover crops also provide OM to soil as exudates in rhizosphere. Gale and Cambardella (2000) and Steenwerth and Belina (2008b) have shown that the contribution of root-C deposition to SOM content and to the consequent stimulation of the microbial metabolism is larger than that produced by soil incorporation of above-ground plant residue. Both living and dead plant cover exhibits a mulching behavior, by reducing soil evaporation, buffering soil temperature (thus reducing SOM oxidation), limiting rainfall impact and transport with runoff, and controlling weeds by soil shading. Uptake of water or nutrients (catch effect) can be only ascribed to plant living cover. More complex effects are linked to pest spread and control, and to other environmental issues such as CO₂ and N₂O emissions (Alluvione et al. 2010). In regions where soil water is the limiting factor, like in Mediterranean area, large emissions from cover crop soils may be restricted to high precipitation periods (Steenwerth and Belina 2008a).

A body of literature concerning soil in temperate regions, and recently in Mediterranean systems, indicates that indirect cover crop effects on SOM, related to reduced need for tillage and fertilization, are as important as direct deposition (Andrews et al. 2002; Hulugalle et al. 2006; Veenstra et al. 2007; Alvaro-Fuentes et al. 2008; Steenwerth and Belina 2008b). SOM increase, in turn, improves soil structure and stability and leads to nutrient retention. Cover crops improve nutrient use efficiency through many mechanisms: provide N through fixation, prevent N losses by inducing a better synchronization of N mineralization and uptake (Drinkwater 2004), help nutrients transfer from year to year, and release them in soil layers where cash crops can take them up more easily (Thorup-Kristensen et al. 2003).

Soil covering also increases organic N availability for mineralization processes, thus reducing the need for N fertilization (Drinkwater 2004). Microbe-mediated processes are also induced by soil mulching and cover crop soils often show a larger microbial N content and a greater capacity for N mineralization, nitrification, denitrification than tilled soils (Steenwerth and Belina 2008b). Effects on crop yield are highly variable: intercropping may result in competition for water and nutrients – especially N – to the point of requiring additional irrigation or fertilization (Skroch and Shribbs 1986). Teasdale et al. (2007) report cash crop yield reductions due to live cover, and regrowth or residues from cut cover crops.

Hairy vetch is largely used as a fall–winter cover in temperate to Mediterranean environments for its rapid establishment in fall and vigorous growth in early spring before planting a macrothermal cash crop. The advantages reside in efficient weed control due to crop planting operations and thick stand, soil protection from fall–winter precipitation, and nitrogen fixation (Teasdale et al. 2007). However, its adoption need to be studied in more detail since weed suppression before harvest is counter-balanced by weed stimulation exerted by decomposing vetch residues, likely because of nitrogen release, as found for *Amaranthus* spp. (Teasdale and Pillai 2005). In general, weed suppression from cover crops appears to be linked to appropriate management rather than to direct effects (Barberi and Mazzoncini 2001).

Experiments on herbaceous cover crops were conducted in Basilicata with the aim of optimizing management for soil protection and yield. In general, grass resulted more efficient in reducing erosion than legumes (Postiglione et al. 1989, 1990), but SOM increases were only found in conjunction with zero tillage and only in the top 10 cm of soil. Pastor et al. (2000) reported SOM and nutrients increases by using cover crops or residue mulching in Mediterranean soils, only in the top 2–5 cm. Living herbaceous covers are intercropped in Mediterranean orchards for erosion control, but in late spring, when precipitations decrease, competition for water may cause yield losses (Pastor et al. 2000). Management options include harvesting the cover crop in early-mid spring and using it for forage or green manure, or simply eliminating it chemically (Pastor et al. 2000). However, trade-offs with reduced soil protection need to be considered with regards to late spring precipitation, and especially to late summer/early fall intense rainstorms, which may be most erosive (Postiglione et al. 1990) especially in absence of soil cover.

Spontaneous living cover was established under olive trees in a 30% sloping soil in Basilicata, and Chiaffitelli et al. (2005) investigated the evolution of potential soil protection after death of cover species in spring. They found that belowground residue decomposition was very low throughout the summer when the soil was dry, and the soil protection from herbaceous root residue was unchanged in early fall. In the same field setting, the lack of N in highly eroded areas favored the colonization of N-fixing cover species, whereas nonfixing species, namely grass, would have been more desirable because of their greater potential for soil protection. The spatial distribution of soil-protecting species was improved after compost application. Chiaffitelli et al. (2005) concluded that managing the complexity introduced by intercropping can enhance the environmental benefits of cover crops and retain

practices oriented toward minimizing yield losses. In Mediterranean environments, it was found that the grass cut in spring and left on soil until fall was more effective than N-fixing species (Chiaffitelli et al. 2005), whose residue undergo faster degradation due to low C/N ratios (Pastor et al. 2000). However, low decomposition rates of surface residue in dry summer conditions imply risk of fire (Pastor et al. 2000), while an excess of mulched residues may interfere with seeding of next fall crops and prevent full crop growth due to toxins release, N immobilization, and alteration of soil temperature and aeration (Teasdale et al. 2007).

3.4.1 Maize Yields

Results from MESCOSAGR field experiments indicate that biomass yield (Table 3.8) from plots green manured with hairy vetch (GMAN) was not generally different from those obtained with mineral fertilization (TRA).

For Torino, treatment effects were concealed by high soil fertility in the first 2 years (total biomass measured in the unfertilized control was not statistically different between GMAN and TRA). In the third year, residual soil fertility was lowered (with yield from unfertilized control soil being lower than for GMAN and TRA), and no difference could be detected in the yield of GMAN and TRA. This suggests that the effect of hairy vetch was not an artifact due to background soil conditions. A similar behavior was observed in the Napoli site from the second year onward. Differently from compost, mineralization of green manure did not limit maize yield in either TO or NA, possibly because vetch biomass is more easily decomposable than compost for its low lignin content (Benincasa et al. 2004; Zavattaro et al. 2003).

3.5 Effects of Organic Matter Amendments

Animal manures and compost can be valuable nutrients sources to crops. In addition, while their amendments to soils represent a convenient disposal and recycle of considerable amounts of wastes, they allow to limit the application of mineral fertilizers and, thus, save farm money and energy. A number of studies have shown that manure addition is beneficial to soil in terms of plant productivity and soil quality (Haynes and Naidu 1998; Edmeades 2003).

A long-term experimental platform exists at the TO site in close proximity of the MESCOSAGR experiment, where organic fertilizers are tested. This platform was intended to evaluate management options of livestock farming in terms of crop production, soil quality, and environment impact. Different maize-based cropping systems are fertilized with bovine farmyard manure or slurry, in comparison to urea. It was therein found that tested organic fertilizers made N available to crops to the same extent as urea and were better retained in the soil leading to minor losses

(Zavattaro et al. 2011). Grignani et al. (2007) showed a lower N efficiency of farmyard manure than that of slurry when supplied at low rates, probably because the available N in farmyard manure was not sufficient at early growth stages. On the contrary, farmyard manure was utilized as well as slurry or even more efficiently (depending on cropping systems) when applied at high rates.

An investigation on chemical and biochemical indicators of soil quality was conducted on a selection of treatments. Farmyard manure was found to increase SOM content, potential mineralizable N and soil microbial biomass. Furthermore, application of farmyard manure was found to store the maximum amount of C and N per unit of C and N received throughout the experimental period. An investigation on soil respiration, under controlled conditions, confirmed that C supplied with farmyard manure was more stable and less decomposable than slurry (Monaco et al. 2008). These authors reported that use of slurry resulted in a lower accumulation of C and N in soil per unit of added C and N than for farmyard manure. With respect to the treatment receiving urea, the liquid manure applications did not change the amount of total N in the soil, but did significantly increase the fraction of easily mineralizable organic N. Data modeling leads to quantify C retention into SOM for 0–30 cm tilled layer. Following these data, after the first year from field application, 26% of slurry-C and 46% of farmyard manure-C is still in soil. Increasing amounts of organic fertilizers enhanced SOM. Conversely, our results showed that increasing amount of urea did not produce any significant difference in SOM content, indicating that the amount of mineral N supplied was not determinant for C sequestration (Bertora et al. 2009).

A research on N mineralization in the same treatments revealed that the time and extent of net N mineralization and plant N uptakes were not affected by fresh manure application. Instead, the effect of past management increased the maximum net N mineralization rate obtained with farmyard manure (Monaco et al. 2010).

Field experiments made in southern Italy in the same site as that of Mescosagr (Fagnano et al. 2011) showed a favorable effect on lettuce yield of compost made with municipal solid wastes (MSW), proving that compost fertilization may have agronomic and environmental benefits in sandy-loam soils, if amendment rates are tuned to N requirements of crops. The compost rate of 30 Mg ha⁻¹ satisfied the N requirements of two lettuce cycles, without causing surplus of nitrogen in postharvest periods and dangerous levels of nitrate and potentially toxic elements in soil and plants. Furthermore, compost fertilization proved to compensate SOM degradation due to cultivation, estimated in 6 Mg ha⁻¹ of C, but was also able to significantly increase SOM content (2.7 Mg ha⁻¹ of C), through C fixation in stable SOM. A study about the effects of such compost on the molecular changes in soil organic C confirmed the formation of more stable C compounds, such as fatty acids, *n*-alkanes, and various biopolyesters derivatives (Spaccini et al. 2009).

3.5.1 Maize Yields

Results from the MESOSAGR project confirmed that compost fertilization produced different crop performances depending on the pedo-climatic conditions. The total yield of plots subjected to fertilization with compost was largely different among sites and presented a large variability over the years (Table 3.8).

In the Torino field site, original soil fertility was large, thus concealing the fertilization effect in the first two experimental years. Conversely, in the third year, the residual soil fertility was significantly depleted, and the effect of compost on soil fertility became detectable. Our results showed that compost treatments can sustain maize production, thus leading to similar yields as for treatments with urea.

In Napoli, intrinsic soil fertility was capable to sustain crop growth only in the first experiment year, when yields of untreated plots were similar to those of plots under mineral fertilization. Maize yield in plots treated with compost were somewhat reduced throughout all experimental years. An explanation may reside in the possible N immobilization induced by compost supply that may have limited N uptake by maize plants. The differences noted between TO and NA may be ascribed to the lower soil aerobicity in NA. In fact, the finer soil texture of the Napoli site may have limited nitrification and, consequently, N uptake by crops (Alluvione et al. 2008). Nevertheless, no significant difference between Torino and Napoli was observed for results from the two compost rates.

Conversely, in the first year of the Battipaglia site, the largest compost rate provided a sorghum biomass production larger than for the untreated plot, while it was not different from that found for plots under mineral fertilization. In the second year, maize yield from compost-treated plots was similar to that from control plots, without significant differences between the two compost rates. In the third year, a severe attack of sorghum soot fly (*Atherigona soccata* Rondani) has lowered yield, thus making impossible to evaluate treatments effects.

3.6 Effects of the Addition of Biomimetic Catalyst on Wheat Trials

A very innovative part of the MESCOSAGR project is the introduction of a soil management practice comprising the soil amendment with a biomimetic catalyst. This practice previews a stabilization and/or improvement of soil organic carbon content by the in situ photo-polymerization of the humic soil fraction (Piccolo et al. 2005; Smejkalova et al. 2006; Gelsomino et al. 2010).

Field results indicate that the treatments with the iron-porphyrin catalyst (CAT) had no effect in Napoli plots on total biomass (Table 3.9) and grain yield. Harvest index was significantly reduced by the Fe-porphyrin treatment only in 2007. The number of kernels per spike and the average weight of kernels did not change in relation to treatment. Nitrogen content of straw was larger in CAT at harvest

Table 3.9 Wheat productive results at the Napoli site

Main factors and interaction	Spikes per m ² (number)	Total biomass (ton ha ⁻¹)	Grain yield (ton ha ⁻¹)	Harvest index (%)	Kernel weight (mg)	Kernels per spike (number)
Treatment						
CAT	258.4b	9.01	2.25b	25.8	54.0	20.5
NO-CAT	310.9a	9.51	2.99a	31.1	54.3	19.9
Significance	0.001	ns	0.001	ns	ns	ns
Year						
2006	363.5a	3.96c	1.45c	31.9a	50.4b	7.6b
2007	194.8c	9.98b	2.50b	25.2c	53.9ab	27.8a
2008	295.7b	13.84a	3.91a	28.3ab	58.1a	25.2a
Significance	0.001	0.001	0.001	0.004	0.019	0.001
Year × Treatment						
2006 CAT	386.7a	4.53	1.69cd	32.8a	54.9ab	7.7
NO-CAT	340.3a	3.39	1.20d	31.0a	45.9b	7.5
2007 CAT	144.7c	9.27	1.78bcd	19.5b	50.9ab	28.2
NO-CAT	245.0b	10.69	3.22abc	30.9a	57.0ab	27.5
2008 CAT	244.0b	13.24	3.28ab	25.2ab	56.3ab	25.6
NO-CAT	347.3a	14.44	4.53a	31.4a	60.0a	24.7
Significance	0.001	ns	0.012	0.005	0.001	ns

Table 3.10 Wheat productive results at the Torino site

Main factors and interaction	Grain yield (ton ha ⁻¹)	Straw yield (ton ha ⁻¹)	Harvest index (%)	Grain N content (%)	Straw N content (%)	Grain N uptake (kg ha ⁻¹)	Straw N uptake (kg ha ⁻¹)
Treatment							
CAT	4.4	10.5	0.30	2.61	1.18	109.7	129.0
NO-CAT	4.0	9.9	0.29	2.56	1.21	98.8	124.9
Significance	ns	ns	ns	ns	ns	ns	ns
Year							
2006	5.9a	7.5b	0.44a	2.18b	0.83c	128.9a	63.2b
2007	3.5b	12.2a	0.22b	2.67a	1.26b	91.5b	154.3a
2008	3.2b	10.8a	0.23b	2.91a	1.50a	92.3b	163.4a
Significance	0.001	0.000	0.000	0.000	0.000	0.003	0.000
Year × Treatment							
Significance	ns	ns	ns	ns	ns	ns	ns

(0.37 vs. 0.33%, significant per $P \leq 0.001$). No negative effect resulted for crops' vegetative phases, since number of tillers per plant and plant heights were not different between un-treated (No-CAT) and treated plants (data not shown). At the Torino site, all measured parameters of crop production resulted not to be influenced by the Fe-porphyrin addition to soil (Table 3.10).

In general, our results of field trials suggest that Fe-porphyrin treatment did not induce any significant effect on the main yield parameters for wheat. The occurred sequestration of carbon in soil upon the CAT treatment (see Chap. 4) did not appear

to have induced any downshift in the N mineralization potentially affecting nitrogen crop uptake, and, thus, productive yields were not different from control.

3.7 Conclusions

Results obtained within the MESCOSAGR project indicate that the proposed innovative soil practices aimed to sequester carbon in soil can be used in southern Europe environmental conditions without affecting crop yields, provided that an appropriate management is adopted.

In particular, it was found that reduced tillage intensity did not affect maize growth in the two contrasting Italian site environments selected for the field trials. Effects of compost application on biomass production were more complex, since crop performances were greatly different among sites and over the three experimental years. In a poorly aerated soil with small mineralization rates, a suppressive effect of compost on maize growth was observed, whereas soils with larger mineralization potential showed that compost can provide enough mineral nitrogen to sustain maize production up to the levels obtained with urea. A rate effect of compost was observed in the first year of application in soil where background fertility was low enough to prevent the masking of crop response to fertilization.

Green manure experiments with hairy vetch indicated that the rapid decomposition of such green residues allowed a crop performance similar to that of urea-treated soils.

The use of a synthetic water-soluble catalyst to promote increased chemical energy in SOM and, thus, carbon sequestration in soil, did not induce any change in wheat yield as compared to control plots. Moreover, we can exclude a reduction in N mineralization, possibly derived from an impeded microbial transformation of SOM, as it clearly arises from the unaltered crop yields.

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Chapter 4

Carbon Sequestration in Soils by Hydrophobic Protection and In Situ Catalyzed Photo-Polymerization of Soil Organic Matter (SOM): Chemical and Physical–Chemical Aspects of SOM in Field Plots

Riccardo Spaccini and Alessandro Piccolo

Abstract The application of innovative practices of soil organic matter (SOM) management, based on either soil amendment with hydrophobic mature compost or with a water-soluble biomimetic catalyst, has shown to enhance organic carbon sequestration in agricultural soils. In three different agricultural sites in Italy, field plots under maize were treated with traditional deep tillage, minimum tillage, green manuring and mature compost, whereas field plots under wheat were added with the iron–porphyrin catalyst. In terms of soil physical quality, mature compost additions improved soil aggregate stability by favoring the increase of water-stable macroaggregates. After 3 years of field experiments, both minimum tillage and green manuring treatments confirmed short-term and even negative effects on organic carbon (OC) accumulation, as compared to traditional tillage. Conversely, the hydrophobic protection exerted by compost amendments on SOM successfully fixed OC in soil from 3 to 22 ton ha^{-1} more than for traditional tillage, depending on the experimental site. Solid-state CPMAS-NMR spectra of humic substances (HS) extracted from treated soils allowed the molecular characterization of the stable organic matter pool. NMR data combined with chemometric methods revealed that compost-added soils progressively incorporated aliphatic and aromatic hydrophobic components into soil humic fractions over the experimental period. Though more variable among experimental sites, the treatment with biomimetic catalyst positively affected both total soil organic carbon (SOC) content and molecular characteristics of humic extracts. An increased aromaticity and hydrophobicity was shown in the spectra of HS from soils treated with the biomimetic catalyst, thus suggesting an effective photo-polymerization of soil aromatic components and their progressive inclusion into the humic pool. In the first and second year of treatments with the water-soluble iron–porphyrin catalyst, the in situ

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catalyzed photo-polymerization of SOM effectively occurred since soils were found to have sequestered from 4 to 24 ton ha⁻¹ more than the control.

4.1 Objectives

Within the MESCOAGR project, two innovative management practices were studied in field experiments (see Chap. 3) to increase OC sequestration in agricultural soils:

1. Soil addition with recycled biomasses, such as mature humified compost, characterized by large hydrophobicity and biochemical stability. This practice is expected to sequester biolabile carbon by hydrophobic protection.
2. Soil treatment with a water-soluble iron–porphyrin biomimetic catalyst (see Chap. 1) to promote an in situ photo-oxidative polymerization of soil organic molecules. The polymerized soil organic matter (SOM) is expected to be less readily mineralized by soil microbes and be fixed permanently in soil.

Our aim was to evaluate the quantitative and qualitative changes brought about in soil organic carbon (SOC) by these two novel techniques, as compared to conventional tillage and other common SOM management practices, such as minimum tillage and green manuring.

The effect of soil management practices on SOC stabilization was evaluated through the following parameters:

- Distribution of water-stable aggregates, and soil structural stability
- OC content in bulk soils and water-stable aggregates
- Physical–chemical characterization of humic substances (HS) extracted from soil

4.2 Soils Characteristics and Experimental Setup

Three different experimental sites along a North–South climate gradient were chosen to set up soil treatments. Soil classification textural composition and organic carbon content of the selected soils are shown in Table 4.1.

The effect of mature compost addition on soil carbon sequestration was evaluated by comparing the following field management during a 3-year experiment (2006–2008), using a monoculture of mais (*Zea mais*) as annual crop (see Chap. 3):

Table 4.1 Textural composition (%), bulk density, and TOC (g kg⁻¹) content of soils from field experiments

Field sites	Soil type	Sand	Silt	Clay	Bulk density	TOC
Torino	Typic Ustifluent	36.9	56.2	6.9	1.50	11.5
Piacenza	Udifuventic Haplustept	17.9	47.1	35.0	1.30	17.2
Napoli	Vertic Haploxeralf	47.0	20.1	32.9	1.40	10.5

- Traditional (TRA): plowing at 35 cm depth, followed by surface harrowing with addition of mineral fertilizers.
- Minimum tillage (MIN): no plowing, with addition of mineral fertilizers.
- Green manure (GMAN): plowing at 30 cm depth, followed by surface harrowing. Leguminous crops were interlaced between two main annual cycles and used as green manure to totally or partially replace nitrogen fertilizer.
- Compost first rate (COM-1): same as the TRA plots but with the addition of an amount of mature compost corresponding to 2.7 ton ha^{-1} of OC.
- Compost second rate (COM-2): same as the TRA plots but with addition of an amount of mature compost corresponding to 5.4 ton ha^{-1} of OC.

Each management treatment was established on a $4 \text{ m} \times 4 \text{ m}$ plot with four replicates, in a randomized block experiment.

The experiment based on the addition of the biomimetic catalyst was performed on $1 \text{ m} \times 1 \text{ m}$ field plots ($n = 4$) cultivated with wheat (*Triticum Durum*) for 3 years, with the following treatments:

- CAT: plowing at 35 cm depth, followed by surface harrowing with the addition of mineral fertilizer and 10 kg ha^{-1} of a biomimetic catalyst, the water-soluble iron–porphyrin (FeP). FeP was synthesized in the laboratory as *meso*-tetra (2,6-dichloro-3-sulfonatophenyl)porphyrinate of iron(III) chloride, Fe-(TDCPPS) Cl (Piccolo et al. 2005a).
- No-CAT: plowing at 35 cm depth followed by surface harrowing with the addition of mineral fertilizers.

4.3 Water-Stable Soil Aggregates and Soil Stability

The dynamics of SOM is closely related to soil physical properties, such as particle sizes distribution and soil aggregation (Tisdall and Oades 1982). Organic matter components are involved in the different steps of soil aggregate hierarchy, from the formation of organo-mineral complexes up to stabilization of aggregate-size classes (Oades and Waters 1991; Six et al. 2004). Since the role of OM in soil physical aggregation determines the processes of OC decomposition and accumulation (Plante and McGill 2002a), an evaluation of aggregate distribution in soil and their stability is useful to follow SOC dynamics (Kasper et al. 2009).

The classical procedure described by Kemper and Rosenau (1986) was used to separate water-stable aggregates. Briefly, 20 g of $<4.75 \text{ mm}$ air-dried soil samples was put on the topmost of a nest of three sieves with 1.00, 0.50, and 0.25 mm mesh size and pre-soaked in distilled water for 30 min. Then, the nest of sieves was oscillated vertically in water 20 times, using a 4 cm amplitude at the rate of one oscillation per second. Care was taken to ensure that soil particles on the topmost sieve were always below the water surface during each oscillation.

After wet-sieving, the water-stable soil materials left on each sieve and the unstable ($<0.25 \text{ mm}$) aggregates were quantitatively transferred into beakers,

dried in the oven at 50°C for 48 h, weighed and stored for analysis. The percentage ratio of aggregates in each sieve represents the water-stable aggregates for size classes: 4.75–1.00, 1.00–0.50, 0.50–0.25, and <0.25 mm. Mean-weight diameter (MWD) of water-stable aggregates was calculated by the following equation:

$$\text{MWD} = \sum_{i=1}^n X_i W_i$$

where X_i is the mean diameter of the i th sieve, and W_i is the amount of total aggregates in the i th fraction.

4.3.1 Control Soils

The distribution of water-stable aggregates obtained from fractionating the initial soils before the start of field experiments revealed a marked influence of textural composition on soil structural properties in the three field sites (Table 4.2). The lowest aggregate stability was found for the silty-loamy soil of Torino that was characterized by both the lowest clay content (Table 4.1) and the largest yield of unstable microaggregates (<0.25 mm). Conversely, the significantly larger stability index (Table 4.2) shown, in the order, by the sandy-clay loam Napoli soil and the silty-clay loam Piacenza soil, was associated with a large amount of clay particles (Table 4.1). No direct relationship could be drawn by relating aggregate stability to bulk OC content (Tables 4.1 and 4.2).

The finest soil mineral components, such as fillosilicates and Fe and Al hydroxides, exert a strong influence on soil structural properties. The large surface area exposed by clay-size particles, in fact, allows a close interaction among inorganic and organic colloidal constituents, and promote formation and stabilization of soil aggregates (Oades and Waters 1991; Attou et al. 1998).

The modifications induced by field treatments on soil aggregate distribution and structural stability during the three experimental years are reported in Tables 4.3–4.5 for the soils of Torino, Piacenza and Napoli, respectively.

Table 4.2 Percent (%) distribution of water-stable aggregates (mm) and mean-weight diameter index (MWD, mm) of initial control soils

Field sites	Aggregate size				MWD
	4.75–1.00	1.00–0.50	0.50–0.25	<0.25	
Torino	9.7	21.1	27.5	41.8	0.59
Piacenza	52.8	26.2	9.1	11.9	1.76
Napoli	39.2	30.8	14.3	15.7	1.43
LSD 0.05	8.5	NS	4.0	3.9	0.18

LSD least significant difference ($n = 4$), NS not significant

4.3.2 Torino Experimental Site

After the first year, the majority of treatments on maize plots of Torino showed a significant increase of aggregate stability with respect to the initial soil (Table 4.3). Only the addition of green manure (GMAN) and the lower rate of compost (COM-1)

Table 4.3 Torino experimental site, percent distribution (%) of water-stable aggregate sizes (mm) and mean-weight diameter index (MWD) (mm) under different treatments for 3 years of experimentation

Treatments	Aggregate size				MWD
	4.75–1.00	1.00–0.50	0.50–0.25	<0.25	
Control soil	9.7	21.1	27.5	41.8	0.59
Maize					
<i>First year</i>					
TRA	15.7	27.1	26.9	30.4	0.79
MIN	13.2	30.9	27.6	28.3	0.75
GMAN	10.8	26.5	31.1	31.6	0.67
COM-1	9.0	27.9	32.0	31.1	0.65
COM-2	14.7	29.3	27.2	28.9	0.78
LSD	1.7	NS	3.2	1.2	0.04
<i>Second year</i>					
TRA	8.8	25.5	39.8	25.9	0.63
MIN	9.6	23.4	38.7	28.2	0.63
GMAN	10.3	24.1	39.7	25.9	0.66
COM-1	9.6	24.1	38.5	27.8	0.64
COM-2	9.7	26.6	39.9	23.8	0.66
LSD	NS	NS	NS	NS	NS
<i>Third year</i>					
TRA	11.4	25.0	36.2	27.5	0.68
MIN	9.8	21.2	38.0	30.9	0.62
GMAN	9.4	19.4	38.3	33.0	0.60
COM-1	15.3	25.2	34.5	25.1	0.79
COM-2	20.3	27.7	29.8	22.2	0.93
LSD	2.5	2.2	2.1	2.2	0.06
Wheat					
<i>First year</i>					
CAT	19.4 (4.2)a	25.0 (4.4)	25.8 (3.8)b	29.8 (8.3)	0.88 (0.11)
No-CAT	12.9 (2.2)b	28.5 (2.5)	32.3 (1.1)a	26.2 (0.9)	0.74 (0.05)
<i>Second year</i>					
CAT	9.5 (1.2)b	23.4 (1.2)	38.2 (3.8)a	28.9 (8.7)	0.64 (0.03)
No-CAT	11.3 (1.7)a	22.5 (1.3)	33.0 (1.7)b	33.1 (2.8)	0.66 (0.04)
<i>Third year</i>					
CAT	10.3 (1.5)	25.0 (1.9)	36.5 (1.4)	28.2 (2.7)	0.66 (0.05)
No-CAT	10.6 (1.4)	24.3 (5.2)	33.9 (3.3)	31.1 (5.4)	0.65 (0.05)

LSD least significant difference ($n = 4$), NS not significant. Different small letters in columns indicate significant difference at 0.05 probability level ($n = 4$). Numbers in brackets for wheat plots represent standard deviation ($n = 4$)

produced smaller MWD increases. All field treatments revealed a positive effect in the distribution of water-stable aggregates, with an overall decrease in the yield of microaggregates (<0.25 mm), which were steadily incorporated in upper size-classes. The widespread increase of soil aggregation may be explained with the physical action of plant roots and fungal hyphae, as well as root debris and microbial bio-products, which promote association of small soil fractions into meso- and macroaggregates (Tisdall and Oades 1982; Chan and Heenan 1999; Six et al. 2004). For the GMAN and COM-1 treatments, the effect was limited to the intermediate particles size fraction (1–0.25 mm), while for traditional (TRA), minimum tillage (MIN), and greater compost rate (COM-2), there was a significant particle incorporation also in the large macroaggregate class, with a consequent increase of MWD.

As compared to both initial (control) and untreated plots (No-CAT), an increase of MWD index (Table 4.3) was observed for the soil added with biomimetic catalyst (CAT), indicating that this treatment induced a redistribution of macroaggregates into larger sized fractions. However, this effect may have also been partially favored by the action of plant roots and microbial biomass.

After the second year, an overall stabilization of structural properties was noted with an even distribution of water-stable aggregates and a similarity of MWD index for all soil treatments. With respect to previous year, a MWD decrease was found for TRA, MIN, and COM-2, whose values approached those for GMAN and COM-1, which instead maintained a steady effect on soil aggregates. The aggregate distribution for the second year (Table 4.3) showed a further decrease of microaggregate sizes for all treatments, with their prevalent incorporation into the next larger size-fraction (0.50–0.25 mm). Concomitantly, the trend of decreasing aggregate stability for TRA, MIN, and COM-2 was mainly due to decrease of large macroaggregates, whose values remained unvaried only for GMAN and COM-1 treatments.

In the second year, soils treated with the biomimetic catalyst (CAT) showed a breakdown of larger aggregates, with soil redistribution in smaller macroaggregates and MWD index returning to No-CAT values.

Soil samples under traditional soil management did not show a significant variation in structural stability after 3 years of experimentation, except for only a slight increase in microaggregate yield (Table 4.3). Conversely, both COM-1 and COM-2 revealed a positive effect on structural properties. In fact, their stability index increased, due to decrease of both microaggregates and small macroaggregates (0.50–0.25 mm), which apparently aggregated into larger sized fractions. This improvement in soil stability was consistent with the amount of added compost, since the larger compost addition (COM-2) provided a greater response. In fact, the yield of large macroaggregates for COM-2 was twice as large as that for TRA, MIN, and GMAN. No difference in the distribution of water-stable aggregates and MWD index was found between the CAT treatment and its control for the third year.

4.3.3 Piacenza Experimental Site

The soil treatments from the Piacenza site showed, throughout the experimental period, a similar trend of soil aggregation as for Torino. An overall improvement of soil aggregation was shown for all treatments after the first year (Table 4.4). This was mainly due to greater yield of large aggregate size fractions, as compared to the initial distribution at the onset of experimentation (initial soil). The major effects were achieved for the least disruptive MIN, COM-2, and CAT treatments. Both soil aggregation and stability index were significantly larger than the respective TRA and No-CAT control treatments.

Table 4.4 Piacenza experimental site, percent distribution (%) of water-stable aggregate sizes (mm) and mean-weight diameter index (MWD) (mm) under different treatments for 3 years of experimentation

Treatments	Aggregate size				MWD
	4.75–1.00	1.00–0.50	0.50–0.25	<0.25	
Control soil	52.8	26.2	9.1	11.9	1.76
Maize					
<i>First year</i>					
TRA	55.6	21.5	11.0	11.9	1.82
MIN	64.3	17.7	9.1	9.0	2.03
COM-2	63.6	18.3	9.3	8.7	2.01
LSD	5.5	NS	NS	1.7	0.10
<i>Second year</i>					
TRA	36.1	33.4	15.6	14.9	1.37
MIN	45.0	27.1	14.7	13.2	1.57
COM-2	45.4	29.5	14.6	10.6	1.59
LSD	3.2	2.4	NS	1.6	0.08
<i>Third year</i>					
TRA	53.7	25.9	10.8	9.6	1.79
MIN	50.4	28.1	11.7	9.9	1.72
COM-2	58.8	24.1	9.3	7.8	1.92
LSD	NS	NS	NS	NS	NS
Wheat					
<i>First year</i>					
CAT	55.1 (0.9)a	23.8 (1.1)	11.2 (0.3)	9.9 (0.3)b	1.82 (0.02)a
No-CAT	49.0 (1.9)b	25.7 (2.7)	10.5 (0.4)	12.5 (1.6)a	1.67 (0.03)b
<i>Second year</i>					
CAT	38.4 (0.1)a	29.9 (0.4)b	16.2 (0.5)b	15.5 (0.8)	1.41 (0.03)a
No-CAT	34.6 (1.5)b	30.8 (0.4)a	17.9 (0.8)a	16.7 (1.0)	1.31 (0.04)b
<i>Third year</i>					
CAT	47.7 (2.9)	28.2 (1.3)	12.5 (0.6)	11.6 (1.4)	1.65 (0.1)
No-CAT	52.5 (5.5)	25.9 (3.0)	11.8 (1.4)	9.8 (1.1)	1.76 (0.1)

LSD least significant difference ($n = 4$), NS not significant. Different small letters in columns indicate significant difference at 0.05 probability level ($n = 4$). Numbers in brackets for wheat plots represent standard deviation ($n = 4$)

A sharp decrease of soil aggregation and stability index occurred in Piacenza for soil treatments under maize after 2 years of field experiments (Table 4.4). All soils showed a breakdown of large size aggregates, and redistribution of soil particles towards smaller size fractions, including microaggregates, though the loss of structural properties was different for various treatments. A persistent effect on soil stability was shown by MIN and COM-2, both characterized by a preservation of water-stable macroaggregates ($\cong 45\%$), and a limited reduction in the associated MWD index. Moreover, aggregate fractionation of COM-2 provided the lowest yield of microaggregates (10.6%), whose amount was even smaller than for the initial control soil (11.9%).

For the wheat plots, despite the overall loss of structural properties in the second year, both size-aggregate distribution and MWD index (Table 4.4) indicated that the treatment with biomimetic catalyst preserved soil structural quality more than for the No-CAT treatment.

The positive effect on aggregate stability for the maize field plots under compost was also confirmed at the end of the third experimental year (Table 4.4). With respect to the results of the previous years, the aggregate distribution of TRA, MIN, CAT, and No-CAT indicated an occurred recovery of the original structural stability. The values of aggregate yields and stability index for these treatments reached those found at the onset of experiments, thereby suggesting a progressive lower efficacy of both MIN and CAT on soil structural quality. Conversely, COM-2 plots showed a continuous decrease of both microaggregates (-2.8%) and intermediate macroaggregate fractions, which became firmly incorporated into larger sized aggregates ($+13.4\%$), thus improving the overall soil structural stability index (1.92).

4.3.4 *Napoli Experimental Site*

Differently from previous experimental sites, the first year of cultivation produced a deterioration of soil structural properties in the field plots of Napoli (Table 4.5). With respect to the initial control soil, nearly all treatments showed lower yields of large size aggregates, with consequent decrease in soil stability index. The loss of structural stability in maize cultivated plots may be due to specific properties of the sandy-clay loam soil of this site. An exception was the soil treated with the low compost rate (COM-1). This showed a MWD increase, due to an effective association of soil particles into larger aggregate fractions, which included more than 50% of total fraction mass. The addition of the low compost rate (COM-1) may have promoted a microbially induced priming effect with a temporary improvement of macroaggregate formation (Chan and Heenan 1999).

In the case of wheat fields, both CAT and No-CAT treatments revealed an improvement of soil structural properties as compared to maize fields, thus suggesting a crop effect on soil physical parameters (Table 4.5).

Table 4.5 Napoli experimental site, percent distribution (%) of water-stable aggregate sizes (mm), mean-weight diameter index (MWD) (mm), under different treatments for three experimental years

Treatments	Aggregate size				MWD
	4.75–1.00	1.00–0.50	0.50–0.25	<0.25	
Control soil	39.2	30.8	14.3	15.7	1.43
Maize					
<i>First year</i>					
TRA	33.3	32.4	16.6	17.6	1.29
MIN	31.9	35.4	18.4	14.2	1.27
GMAN	28.7	39.8	16.8	14.7	1.20
COM-1	53.6	26.9	9.5	10.0	1.79
COM-2	37.3	31.4	12.7	18.6	1.38
LSD	3.3	1.1	3.7	4.7	0.19
<i>Second year</i>					
TRA	26.4	40.1	20.1	13.4	1.15
MIN	33.1	41.6	15.8	9.5	1.33
GMAN	32.4	38.4	17.3	11.9	1.30
COM-1	37.6	37.2	14.9	10.4	1.42
COM-2	32.6	36.5	18.1	12.8	1.30
LSD	5.3	3.7	2.1	1.2	0.10
<i>Third year</i>					
TRA	38.7	33.7	16.1	11.5	1.44
MIN	40.8	31.9	16.1	11.2	1.49
GMAN	46.1	31.4	13.2	9.3	1.62
COM-1	43.1	29.9	15.5	11.5	1.54
COM-2	43.2	32.6	14.7	9.5	1.55
LSD	2.2	NS	NS	2.4	0.06
Wheat					
<i>First year</i>					
CAT	51.3 (2.4)	25.9 (1.9)	11.2 (2.3)	11.6 (1.7)	1.73 (0.06)
No-CAT	47.5 (7.3)	28.2 (4.3)	13.3 (2.0)	10.9 (2.7)	1.64 (0.18)
<i>Second year</i>					
CAT	31.5(2.7)	40.0(0.4)	17.1(1.7)	11.4(1.5)	1.28(0.1)
No-CAT	36.7(6.0)	40.2(3.0)	13.2(1.9)	9.9(1.1)	1.42(0.1)
<i>Third year</i>					
CAT	41.6(1.9)	32.7(0.3)	15.2(0.6)	10.4(0.9)	1.51 (0.05)
No-CAT	39.7(2.2)	35.0(1.4)	15.2(0.5)	10.1(0.4)	1.47 (0.05)

LSD least significant difference ($n = 4$), *NS* not significant. Different small letters indicate significant difference at 0.05 probability level ($n = 4$). Numbers in brackets for wheat plots represent standard deviation ($n = 4$)

A slight different distribution of water-stable aggregates was observed in the maize plots after two experimentation years. For all treatments, soil aggregate fractionation indicated a decrease in microaggregate yield and a corresponding, though uneven, increase in macroaggregates, this trend being more effective for COM-1 (Table 4.5). For maize fields, soil aggregate distribution decreased the

MWD index, with respect to the first year, for all treatments except for MIN and GMAN. The MWD stability index for both CAT and No-CAT treatments was also reduced in the second year to the levels similar to those of maize fields.

Similar to other experimental sites, the Napoli field site presented a structural improvement in soils of all treatments after three experimental years (Table 4.4). Either maize or wheat plots produced an increased amount of large macroaggregates fraction, with a consequent improvement of MWD values, which were larger than for the initial undisturbed control soil.

The best structural quality was found for treatments with organic amendments, including green manure, and for CAT, although for the latter the difference from No-CAT was not statistically significant (Table 4.5).

4.3.5 Concluding Notes on Soil Physical Quality

As mentioned earlier, the yields of aggregate fractionation and values of soil stability index suggested a predominant influence of specific texture and clay content on the physical and structural properties of soils. However, all treatments in each site, despite the specific soil structural properties, showed a comparable macroaggregate dynamics throughout the experimental period, consisting first in a decrease and then in a recovery of soil stability. In fact, our results revealed a similar succession of aggregate yields and stability index, whose values varied annually around the average level of the respective undisturbed initial soils.

The effectiveness of clay particle sizes, as binding agent, is strongly related to soil physical–chemical conditions and imposed mechanical stress. Soil pH, ionic strength, and salt composition of soil solution, water holding capacity, freezing/thawing and wetting/drying cycles, raindrop impact, and intensity and frequency of soil tillage, may temporarily overcome the glueing effect exerted by mineral colloids on soil aggregates. In cultivated soils with low SOM content, the lower resilience of clay particles may result in a slow response to the aggregation capacity of soil management (Spaccini et al. 2004), and a significant loss of structural stability for heavy textured soils. In fact, the susceptibility of cultivated soils to loose structural stability is a function of initial aggregation, that is greater for stable clayey than for fragile sandy soils (Spaccini et al. 2001).

Moreover, large soil aggregates, placed in the upper level of aggregate hierarchy, are usually characterized by large porosity, wide planes of weakness, and low tensile strength (Oades and Waters 1991). Therefore, macroaggregates are mostly affected by land use and soil disturbance, and undergo rapid turnover cycles and fast aggregation/disaggregation dynamics, especially for agricultural soils with low OC content (Plante and McGill 2002b). Since turnover time of macroaggregates ranges from 10 to 90 days (Plante et al. 2002; De Gryze et al. 2006), with significant fluctuation during growing seasons, more frequent samplings should better show the effect of management practices on soil aggregation (Daraghmeh et al. 2009). Thus, since the present experiment was mainly focused on the cumulative effects of

soil management on SOC, the unique sampling date at the end of the growing season may have partly masked the differences among soil treatments.

Nevertheless, though results indicate a small effect of soil management as compared to intrinsic soil physical properties, significant differences on aggregate-size distribution and MWD were found among various treatments in the three sites. A similar aggregation process was revealed by fractionating TRA, MIN, and GMAN soils at any experimental site. This indicates that either a reduced soil disturbance by minimum tillage, or green manuring by soil incorporation of residues from leguminous crops, did not significantly modify the aggregate dynamics, neither in the sandy-loam soil of Torino, nor in the heavier textured soils of Piacenza and Napoli. On the contrary, a slight but significant improvement of soil aggregation and structural stability were found for both compost treatments (COM-1 and COM-2), in comparison to either TRA, MIN, or GMAN.

Contrasting results have been reported on the relation between soil aggregate stability and green manuring, when in combination with either reduced tillage or conventional tillage (Biederbeck et al. 1998; Podwojewski and Germain 2005). The main purpose of green manuring relies in the supply to soil of a slow-release source of organic nitrogen, in order to reduce or even replace mineral fertilization. The organic residues added with green manuring are easily decomposable and low in lignified tissues and hydrophobicity (Carvalho et al. 2009). These biolabile and hydrophilic characteristics are recognized to provide at best only a transient effect on soil stability (Piccolo and Mbagwu 1999).

Current findings on soil management methods based on no-tillage practices (NT) indicate an overall improvement of soil physical properties and soil stability, as compared to conventional tillage (CT) (Six et al. 2004). On the other hand, the main effects of reduced tillage methods on soil aggregation are limited to surface horizons, while small differences from conventional tillage are usually found below 10 cm with increasing plow depth (Liebig et al. 2004; Kasper et al. 2009). This emphasizes the importance of investigating the whole soil profile when studying the suitability of NT versus CT for aggregate stability and SOC sequestration (Plaza-Bonilla et al. 2010).

A large literature indicates that soil amendments with different compost materials provide an effective improvement in aggregate stability and related physical properties (porosity, infiltration rate, surface erosion, etc.), regardless of soil type and crop rotation (Weber et al. 2003, 2007; Sodhi et al. 2009). Although soil structure improvement is closely associated with an increase of organic carbon content, the quality and molecular composition of added organic matter play a basic role in the long-term stabilization of soil aggregates (Piccolo and Mbagwu 1999). Soil amendments with compost may promote two possible effects on soil physical quality (1) a transient or temporary soil aggregation through the stimulation of microbial activity and consequent production of stabilizing exopolysaccharides, and, (2) a long-term stabilization due to addition to soil of an effective amount of hydrophobic humified materials (Albiach et al. 2001; Romàn et al. 2003; Bifubusa et al. 2008). In fact, depending on the type and quality of compost, its amendment to

soils may produce either short term, poor, or even negative effects on soil aggregate stability (de León-González et al. 2000; Kohler et al. 2008).

As reminded above, the transient effect observed on soil aggregates of COM-1 field plots in the Napoli site in the first year (Table 4.5) may be related to an increased amount of microbial bio-products due to a priming effect on SOC. This explanation may be supported by the low OC amount found in bulk soil and large soil aggregates (Table 4.8). However, the results on aggregate-size distribution and stability index reported for COM-1 and COM-2 for all field sites after 3 years (Tables 4.3–4.5) indicate that the progressive incorporation of humified and hydrophobic organic matter from compost allowed a slow but persistent improvement of soil aggregation. The largest effect was found for Torino, where the intrinsically low initial soil structural stability was more easily improved by stable compost (Piccolo et al. 2004), and favored a significant incorporation of fine aggregate sizes into larger stable aggregates.

4.4 Organic Carbon in Bulk Soil and Water-Stable Aggregate-Sizes

Assessing the content of total SOC by an elemental analyzer (Interscience EA1108) is the simplest and most direct measurement to evaluate the effect of soil management practices and cropping systems on SOC accumulation or decomposition. However, an estimate of the total amount of organic carbon (TOC) in the bulk soil does not inform on the mechanism of SOM incorporation, but it is only an indirect evaluation of both SOM dynamics and stabilization process.

A large number of chemical or physical fractionation methods have been applied to SOM studies in order to quantify organic matter pools with different OC turnover time and dynamics. The separation of various SOC fractions, based on both SOM physical protection and biochemical recalcitrance, was generally achieved by fractionation of soil particle sizes or aggregates (Gregorich et al. 1989; Angers et al. 1995; Puget et al. 2000; Six et al. 2004), density flotation of aggregates and organic mineral complexes (Christensen 1992; Gregorich et al. 1997; Janzen et al. 1992), chemical separation (Spaccini et al. 2000; Derenne and Largeau 2001; Helfrich et al. 2007), and a combination of physical and chemical methods (Spaccini et al. 2001; Leifeld and Kögel-Knabner 2005; Quénée et al. 2006). Although each technique may provide information to clarify SOM accumulation processes, most of the applied methodologies show different specificities according to soil and procedure. Thus, no common protocol has been yet established for univocal or unambiguous determination of SOM pools and fractions (Smith et al. 2002; von Lützow et al. 2007).

In the field experiments of the MESCOSAGR project, we focused on the determination of OC content in both bulk soils and water-stable aggregate sizes. Moreover, we appraised the molecular composition of humic substances which

were annually extracted from soil after each crop cycle during the three experimental years (see Sect. 4.5).

The TOC content and its distribution among soil aggregates showed the short- and medium-term effects of various field treatments on OM incorporation in soil and its interactions with soil particles. On the other hand, the structural characterization of humic substances extracted from soil indicated the effect of field experiments on the molecular composition of the most stable soil organic components, and the potential of different management for a long-term stabilization of SOM.

4.4.1 *Torino Experimental Site*

After 1 year of maize cultivation, TOC values in bulk soils (Table 4.6) did not reveal significant differences among treatments, except for MIN, that showed the lowest OC content with a decrease of about 1 g OC kg^{-1} with respect to TRA. The addition of exogenous organic matter (GMAN, COM-1, and COM-2) did not produce any increase with respect to TRA. This implies that the fresh organic matter stimulated a microbial mineralization that may have counteracted the OC addition to soil.

With the exception of COM-2, the OC distribution in soil aggregate sizes generally showed a progressive larger OC absolute content with increasing aggregate size, while the relative (%) OC amount was mainly related to the aggregate mass distribution. The OC increase in larger soil aggregates is in line with the theoretical organization of aggregate hierarchy, by which macroaggregates become richer in OC due to the progressive mutual association of fine particles in larger aggregates (Puget et al. 1995; Six et al. 2004). The large OC found in the 0.50–0.25 mm aggregate size in COM-2 may be explained with a compost-induced greater microbial activity, whose bio-products are preferentially adsorbed on the large surface areas of fine aggregate sizes (Zech and Guggenberger 1996). This finding is in line with those for GMAN and COM-1, which also showed, with respect to TRA and MIN, the lowest relative (%) OC in large aggregates (4.75–1 mm), and greater absolute and relative OC content in small aggregate sizes (0.50–0.25 and $<0.25 \text{ mm}$).

For wheat fields (Table 4.6), a greater OC content in bulk soil was found for plots treated with the water-soluble iron–porphyrin catalyst (13.2 g kg^{-1}), as compared to control plots (12.4 g kg^{-1}). However, aggregate-size fractionation provided a low OC recovery for both CAT and No-CAT treatments, with 91 and 93% of TOC recovered for bulk soil, respectively (Table 4.6). This result suggests a loss of soluble organic matter due to a weaker interaction between organic components and surface of mineral particles. Since these values were lower than those obtained for the same soil under maize, the reason cannot be attributed to differences in soil physical properties, but possibly to a different OC incorporation depending on both treatment and crop. The OC distribution in water-stable aggregates for CAT

Table 4.6 Torino experimental site, amount (g kg^{-1}) and relative distribution (%) of organic carbon in bulk samples and water-stable aggregate sizes (mm) in soil under different treatments for 3 years of experimentation

Treatments	Bulk g kg^{-1}	Aggregate sizes						Sum of fractions			
		4.75–1.00		1.00–0.50		0.50–0.25		<0.25		g kg^{-1}	%
		g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%		
Control soil	11.5	12.1	11.0	10.0	19.9	11.1	28.8	10.3	40.4	10.6	92.2
Maize											
<i>First year</i>											
TRA	11.6	13.6	19.2	11.9	29.1	10.4	25.3	9.6	26.4	11.0	94.8
MIN	10.7	10.9	14.3	10.2	31.0	10.0	27.2	9.8	27.5	10.1	94.4
GMAN	11.8	11.6	11.2	11.9	28.0	11.5	31.9	10.3	29.0	11.3	95.8
COM-1	11.8	13.1	10.2	12.2	27.7	11.8	32.9	10.2	29.4	11.5	97.5
COM-2	11.4	9.2	12.2	11.5	30.1	12.2	29.6	11.0	28.1	11.2	98.2
LSD	0.45	1.0	0.6	1.1	2.4	0.6	1.9	0.2	NS	1.0	
<i>Second year</i>											
TRA	11.5	13.4	10.9	10.9	26.1	10.4	38.9	9.9	24.1	10.7	93.0
MIN	11.5	17.2	14.2	14.4	28.8	10.0	33.0	10.0	24.2	11.3	98.2
GMAN	10.8	11.8	11.3	10.9	24.3	11.4	42.2	9.3	22.3	10.7	99.0
COM-1	12.1	11.4	9.0	10.2	20.3	13.8	43.7	11.7	26.9	12.1	100
COM-2	12.4	14.9	11.5	13.9	26.2	13.1	41.0	9.4	21.3	12.4	100
LSD	0.50	1.5	0.6	1.7	1.6	0.7	1.9	0.8	1.2	0.8	
<i>Third year</i>											
TRA	11.6	12.0	11.7	11.9	25.8	11.7	36.7	10.8	25.8	11.5	99.1
MIN	11.3	11.5	9.9	11.5	21.3	11.4	38.2	11.2	30.5	11.4	100
GMAN	12.0	12.2	10.1	11.5	19.8	11.4	38.7	10.7	31.3	11.3	94.2
COM-1	12.7	14.6	17.7	13.4	27.0	11.5	31.6	12.0	23.9	12.6	99.2
COM-2	13.2	15.0	23.1	15.1	31.8	12.0	27.1	10.7	18.0	13.2	100
LSD	0.40	0.9	0.7	0.9	1.3	0.4	1.4	0.7	1.0	0.7	

revealed a generally larger OC content in greater aggregate size fractions than for No-CAT, though a significant difference from No-CAT was found only for the 0.50–0.25 mm aggregate size. This microaggregate size-fraction is composed of fine particles (silt and clay) (Six et al. 2004) mutually associated in organo-mineral complexes due to interactions exerted by adsorbed aliphatic and aromatic components (Baldock and Skjemstad 2000; Spaccini et al. 2002). Thus, it is likely that the catalyst added in the CAT treatment has favored the covalent coupling of aromatic and phenolic SOM components, thus further stabilizing the organic constituents adsorbed on this fine soil fraction.

After two experimentation years, both COM treatments determined an increase of TOC in bulk soils, whereas a decrease was observed for GMAN (Table 4.6). Although GMAN may represent an important supply for plant N requirements, the fresh organic matter added to soil with this treatment may not produce a stable incorporation of organic material (Scholes et al. 1997; Puget and Drinkwater 2001; Spaccini et al. 2004). Conversely, with respect TRA, about 0.6 and 1.0 g kg⁻¹ of OC were additionally incorporated in COM-1 and COM-2 bulk soils, respectively. Based on plowed soil depth (0.35 m) and average soil bulk density of 1.4 (Table 4.1), the OC fixed corresponded to about 0.6 and 1.1 g kg⁻¹ for COM-1 and COM-2, respectively. Therefore, at the end of the second year, the COM plots retained around 100 and 90% of OC added with compost. This means that, by comparing the OC content in MIN and COM-1 between first and second experimentation year (Table 4.6), the SOC fraction in COM-1 must have inherited an aliquot of organic matter incorporated in the first year. The MIN treatment was instead able to significantly fix OC during the second crop cycle, thus recovering the difference from TRA observed in the first year.

The OC distribution in water-stable aggregates after the second year showed that the relative OC content was still related to the physical fractionation yield (Table 4.6). An increase of OC content in large aggregate sizes was shown by TRA, MIN, and COM-2, whereas a greater OC concentration in smaller aggregate sizes was found in GMAN (0.50–0.25 mm) and in COM-1 (0.50–0.25 and <0.25 mm), thus suggesting an accumulation of microbially derived organic components in the latter treatments. The low OC recovery after aggregate fractionation of TRA (92.8% of OC in bulk soil) may imply a contribution of coarse debris from crop residues to TOC of bulk soil. The incorporation of fresh plant-derived organic material was suggested by the large OC concentration (17.4 g kg⁻¹) found in MIN macroaggregates (4.75–1.00 mm), thus indicating that little soil disturbance in MIN may have favored the inclusion of particulate organic matter in the building up soil aggregates (Angers et al. 1995; Puget et al. 1995; Angers and Giroux 1996; Six et al. 2000).

Despite the low OC recovery in the aggregate fractionation of wheat soils after two experimentation years (Table 4.6), an OC fixation was evident in CAT due to amendment with biomimetic catalyst. Both absolute and relative OC contents in CAT water-stable aggregates showed a preferential OC incorporation in larger size-fractions.

After three experimentation years, the TOC content showed that both COM-1 and COM-2 were able to increase OC with respect to other maize treatments. In comparisons to the first year, when no difference was observed among different treatments, COM-1 and COM-2 revealed, at final experimental time, an additional OC content of about 0.9 and 1.8 g kg⁻¹, respectively, corresponding to the 87% of the total averaged organic carbon added with compost in the second and third year of soil treatment.

Also GMAN showed a 1.2 g kg⁻¹ increase of TOC content in the third year, as compared to the second year. For an average OC content of about 55% in leguminous plants, this additional amount in soil corresponded to about 13 ton ha⁻¹ of green manure material, and implied that the totality of leguminous plants added to soil as GMAN should have been retained. However, leguminous crops are reckoned to rapidly mineralize, once incorporated in soil (Fernandes et al. 1997; Spaccini et al. 2004), and hardly contribute to stable SOM (Scholes et al. 1997; Puget and Drinkwater 2001). Hence, the larger TOC found in GMAN should be mainly accounted to maize crop residues left on soil.

The OC content in soil aggregates of COM plots after 3 years indicates the capacity of this treatment to increase SOM (Table 4.6). All field treatments under maize revealed an increased OC in large aggregate sizes. However, the greatest absolute and relative OC content was found in the >0.50 mm aggregate class for both COM treatments. This confirms the effectiveness of hydrophobic humified matter in improving soil aggregation and stability, as already suggested by mass yield of soil fractionation (Table 4.2). Conversely, the lower OC recovery in soil aggregate size fractions for GMAN further indicates the poor OC stabilization conferred by this treatment that prevalently provides easily biolabile organic matter in soil.

No significant difference was found between CAT and No-CAT after 3 years for TOC content in bulk soils (Table 4.6). However, the OC distribution in separated aggregate sizes clearly indicates that SOM stabilization was induced by the biometric catalyst treatment. In fact, the 0.50–0.25 and <0.25 mm aggregate size fractions revealed a significant OC increase due to the catalyst, reaching 14.0 and 14.9 g kg⁻¹ for CAT, and only 11.1 and 10.0 g kg⁻¹ for No-CAT, respectively. This further suggests that the catalyzed photo-polymerization of organic constituents of SOM had occurred in situ and its products were associated with the finest soil particle fractions.

4.4.2 Piacenza Experimental Site

After 1 year of maize cultivation, all field treatments showed an overall TOC depletion in bulk soils (Table 4.7). Similar to Torino, the largest decrease was found for MIN, while a lesser OC reduction was found for TRA and COM-2 (Table 4.7). Also for Piacenza, the failure of compost amendment to enhance SOM content in the first year may be attributed to an extensive priming effect induced by compost organic matter.

Table 4.7 Piacenza experimental site, amount (g kg^{-1}) and relative distribution (%) of organic carbon in bulk samples and water-stable aggregate sizes (mm) in soil under different treatments for 3 years of experimentation

Treatments	Bulk g kg^{-1}	Aggregate sizes						Sum of fractions			
		4.75–1.00		1.00–0.50		0.50–0.25		<0.25		g kg^{-1}	%
		g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%		
Control soil	17.2	15.8	54.0	15.7	26.8	14.4	8.5	14.0	10.7	15.4	89.5
Maize											
<i>First year</i>											
TRA	16.7	12.7	49.1	11.1	16.5	23.4	17.9	20.2	16.5	14.4	86.2
MIN	14.5	14.3	65.5	12.7	15.9	14.3	9.2	14.7	9.4	14.0	96.6
COM-2	15.1	12.2	51.8	9.0	11.0	35.5	22.0	26.0	15.2	15.0	99.3
LSD	1.27	1.0	1.0	1.6	0.9	3.5	0.7	2.5	0.7	NS	
<i>Second year</i>											
TRA	16.6	17.6	43.7	10.4	23.8	15.5	16.4	15.7	16.0	14.6	87.9
MIN	16.3	16.5	46.5	17.4	29.5	14.5	13.3	13.0	10.7	16.0	98.1
COM-2	17.0	14.8	40.1	15.0	26.5	20.6	18.0	24.3	15.4	16.7	98.2
LSD	0.70	1.0	0.9	0.7	0.3	2.6	0.4	1.7	0.8	0.4	
<i>Third year</i>											
TRA	18.7	16.7	50.5	16.2	23.8	27.9	16.9	16.8	8.8	17.6	94.1
MIN	22.4	22.3	52.1	21.4	27.8	20.7	11.2	19.8	8.9	21.5	96.0
COM-2	23.5	23.3	60.4	20.1	21.4	25.3	10.4	22.8	7.8	22.7	96.6
LSD	1.30	2.8	1.5	1.8	0.3	5.2	0.6	5.5	0.2	0.7	
Wheat											
<i>First year</i>											
CAT	16.7 (0.4)	16.2a (0.1)	54.4a (0.9)	16.0 (0.4)	23.2b (0.2)	18.1 (0.1)	12.4 (0.1)	16.7a (0.1)	10.0b (0.1)	16.4 (0.4)	98.2
No-CAT	16.3 (0.5)	15.3b (0.4)	49.1b (0.3)	15.6 (1.1)	26.2a (0.4)	18.1 (0.9)	12.4 (0.3)	15.1b (0.5)	12.3a (0.4)	15.3 (0.1)	93.8
<i>Second year</i>											
CAT	13.4a (0.1)	11.4b (0.2)	33.4b (0.3)	12.2a (0.1)	27.8b (0.3)	19.0a (0.5)	23.3a (0.5)	13.2a (0.3)	15.5 (0.1)	13.2a (0.5)	98.5
No-CAT	12.2b (0.3)	13.2a (0.3)	37.3a (0.5)	11.7b (0.2)	29.3a (0.1)	12.3b (0.5)	18.0b (0.1)	11.3b (0.3)	15.4 (0.1)	12.2b (0.4)	100
<i>Third year</i>											
CAT	19.4a (0.5)	17.3 (0.8)	43.5b (0.1)	18.0 (0.8)	26.7a (0.1)	26.1a (0.8)	17.2a (0.3)	20.9a (2.1)	12.6a (0.1)	19.0a (0.2)	97.9
No-CAT	17.0b (0.9)	16.5 (0.6)	52.2a (0.5)	16.4 (1.2)	25.6b (0.1)	17.5b (1.6)	12.4b (0.1)	16.7b (1.6)	9.9b (0.1)	16.5b (0.4)	97.0

LSD least significant difference for p 0.05 ($n = 4$), NS not significant. Numbers in brackets for wheat plots represent standard deviation ($n = 4$). Different small letters in columns indicate significant difference at 0.05 probability level ($n = 4$)

However, the experiment's progression in the following 2 years indicates that an increasing trend of OC content in bulk soils was shown by both MIN and COM-2, as compared to TRA. In fact, both MIN and COM-2 had a significant larger OC values than TRA in the third year.

Contrary to Torino, a larger OC content was generally found, throughout the experimentation, for all maize treatments in the smaller 0.50–0.25 and <0.25 mm aggregate fractions (Table 4.7). This result is attributed to the influence of physical properties in the soil aggregate hierarchy and, particularly, of the role played by soil texture. Therefore, the large mass found in macroaggregates for the heavy-textured control soil of Piacenza (Tables 4.1 and 4.4) was somewhat reduced over the years in the deeply plowed TRA and COM-2 treatments, with the latter showing the greatest OC content in the fine size fractions (Table 4.7). On the other hand, the least disturbed MIN treatment kept a OC distribution in size fractions similar to the control Piacenza soil. These findings were further supported by data on the relative (%) amount of OC in soil aggregate sizes, which were strongly correlated to mass distribution of the same size fractions (Table 4.4). Moreover, the difference in OC stabilization among treatments was also revealed by the low OC recovered from fractionation of TRA soil (86%), thus indicating an unstable incorporation of free coarse organic debris in the water-stable aggregates of this soil.

As for wheat plots, while no effect of biomimetic catalyst was observed in bulk soils in the first year as compared to control (No-CAT), OC increased significantly in CAT bulk soils in both the second and third year (Table 4.7). Moreover, a significant increase in OC was found in the sum of size fractions of CAT, as compared to No-CAT, for both the second and third year. This was the consequence of the significantly larger OC content found in the smallest <0.25 mm size fraction of CAT, as compared to No-CAT. Since the association of very fine soil particles promotes the formation of new microaggregates also in already existing macroaggregates (Oades 1984; Six et al. 2004), this result may be explained with the catalyst effect on soil organic components and their association in recently formed microaggregates.

4.4.3 *Napoli Experimental Site*

After the first experimentation year, the OC content in Napoli bulk soil decreased in TRA, MIN, and COM-1 to 8.9, 9.8, and 9.5 g kg⁻¹ values, respectively, as compared to the 10.5 g kg⁻¹ level of control soil (Table 4.8). A maintenance of the initial OC content was shown by GMAN (10.5 g OC kg⁻¹), while additional organic carbon (11.3 g OC kg⁻¹) was incorporated in the soil by the COM-2 treatment. This result seems to indicate that also in the Napoli site a priming effect induced by COM-1 promoted the mineralization of previously incorporated OM.

The findings observed for bulk soils were confirmed by the OC content found in water-stable aggregates (Table 4.8). The soil fractionation of TRA, MIN, and GMAN followed the typical hierarchical distribution of aggregates with decreasing

Table 4.8 Napoli experimental site, amount (g kg^{-1}) and relative distribution (%) of organic carbon in bulk samples and water-stable aggregate sizes (mm) in soil under different treatments for 3 years of experimentation

Treatments	Bulk g kg^{-1}	Aggregate sizes						Sum of fractions			
		4.75–1.00		1.00–0.50		0.50–0.25		<0.25		g kg^{-1}	%
		g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%		
Control soil	10.5	11.6	43.3	10.5	30.7	9.3	12.7	8.9	13.3	10.5	100
Maize											
<i>First year</i>											
TRA	8.9	9.4	37.5	7.8	30.7	8.2	16.5	7.2	15.2	8.2	92.0
MIN	9.8	10.4	34.1	9.9	36.0	9.0	17.0	8.8	12.8	9.8	100
GMAN	10.5	11.4	32.2	10.3	41.0	8.9	14.8	8.2	12.0	10.1	96.2
COM-1	9.5	8.4	49.8	9.6	27.6	11.4	11.3	11.1	11.3	9.4	98.9
COM-2	11.2	11.4	37.3	11.3	31.0	9.3	10.2	13.3	21.4	11.4	100
LSD	0.5	1.5	1.7	0.5	1.9	1.4	1.8	1.9	2.0	0.40	
<i>Second year</i>											
TRA	9.1	9.5	27.7	8.6	38.6	9.8	22.0	7.8	11.7	8.9	97.8
MIN	10.3	11.6	36.8	10.5	42.0	9.2	13.9	7.8	7.2	10.3	100
GMAN	10.0	10.1	35.3	9.4	39.2	8.2	15.4	7.7	10.0	9.2	92.0
COM-1	10.0	10.7	41.3	9.2	34.9	9.6	14.7	8.6	9.2	9.8	98.0
COM-2	11.3	12.6	36.9	11.3	37.1	9.7	15.8	8.9	10.2	11.1	98.3
LSD	0.5	0.6	1.1	0.5	1.0	0.6	1.8	0.8	1.3	0.60	
<i>Third year</i>											
TRA	9.5	10.0	44.4	7.8	30.4	8.5	15.6	7.2	11.1	8.7	91.6
MIN	10.1	10.1	44.0	9.2	31.3	9.2	15.8	7.5	10.8	9.4	93.1
GMAN	10.0	9.4	44.9	7.9	25.8	12.0	16.6	13.0	10.2	9.6	96.0
COM-1	10.5	10.1	41.9	11.8	33.9	10.9	16.6	7.2	11.1	10.2	97.1
COM-2	12.5	12.2	42.5	11.8	31.2	13.1	15.6	13.9	12.2	12.4	99.2
LSD	0.5	0.5	1.7	0.9	1.5	0.4	NS	1.2	0.5	0.60	
Wheat											
<i>First year</i>											
CAT	10.4 (0.1)	10.9 (0.2)	54.6a (1.1)	9.6b (0.3)	24.3b (0.2)	10.3 (0.7)	11.1b (0.8)	9.7 (0.3)	9.9 (0.3)	10.2 (0.3)	98.1

OC towards smaller aggregates. Conversely, a significantly larger OC content was found in the 0.50–0.25 mm size fractions for COM-1 and in microaggregates (<0.25 mm) for both COM-1 and COM-2. As already reminded, this result may be attributed to adsorption of microbially processed organic components on fine soil particles and their association in microaggregates. While COM-2 appeared to better incorporate organic carbon in water-stable aggregates, the low OC recovery from fractionation of the TRA soil (92%) suggests that its OC content was due mainly to hardly transformed and, thus, coarse incorporated plant residues.

For soils under wheat, no consistent differences in OC content were found between CAT and No-CAT for either bulk soils or water-stable aggregates (Table 4.8), thus showing that the biomimetic catalyst did not stabilize SOM components in the first year.

Some differences in SOM dynamics among treatments were shown in Napoli after the second experimental year (Table 4.8). An increase of about 0.5 g kg^{-1} in bulk soil OC, as compared to the first year, was noted for MIN and COM-1. The same OC level found in TRA (9.1 g kg^{-1}) and COM-2 (11.3 g kg^{-1}) indicates that both OC deposition by crop in subsoil and OM added with compost were able to maintain the SOC content of the first year. Conversely, the plant residues mixed in soil with the GMAN treatment were not similarly effective and TOC content in this bulk soil decreased in this treatment.

A stable OM incorporation in aggregate size fractions was suggested after the first year in MIN, COM-1, and COM-2 by the large OC recovery in sum of fractions of these treatments. The OC stabilization was particularly evident for both COM rates which showed a significantly greater OC content in <0.25 mm microaggregates (Table 4.8). On the other hand, the low OC recovery in GMAN sum of fractions (92.4%), with respect to the OC of bulk soil, further suggests a weaker interaction between soil aggregates and organic matter added by green manuring.

The treatment with the biomimetic catalyst resulted more effective in the second year of wheat cultivation (Table 4.8). TOC content in CAT bulk soil increased about 1.0 g kg^{-1} with respect to the first year, while that of No-CAT was reduced by 1.5 g kg^{-1} . The effectiveness of SOM stabilization promoted by catalyst addition was further indicated by the OC distribution in water-stable aggregates. An increased absolute OC content was found in <1.0 mm aggregate sizes in CAT soil. However, the relative % OC distribution in the 4.75–1.00 mm size fraction was also significantly larger than for No-CAT. These results are in line with those found for wheat plots in Torino and Piacenza, for which OM was preferentially associated with the finest soil particles due to the catalyzed coupling of humus aromatic compounds.

The superior OM fixation in soils treated with compost and biomimetic catalyst, with respect to conventional management practices, was revealed by the OC content in bulk soils and aggregate size fractions of the third year (Table 4.8). Bulk soils under COM-1 and COM-2 showed an OC content larger than that of second year by 0.5 and 1.2 g kg^{-1} , respectively, and even 1.0 and 2.5 g kg^{-1} greater than for TRA of the same third year. As for COM-1, the final OC value in bulk soil (10.5 g kg^{-1}) was the same as that of the initial undisturbed soil. However,

although the priming effect noted in all soils with this treatment caused SOM losses, the OC incorporated in the Napoli site by COM-1 over control corresponded to $0.5 \text{ g kg}^{-1} \text{ year}^{-1}$. Based on an average 1.4 soil bulk density and 0.35 m plow depth, this amount represents about 80% of the total OC added with COM-1 in the 2 years of experimentation. This consideration, along with the observed large OC fixed by COM-2, confirms that mature compost amendment in the conditions of the Napoli site appears as a reliable method for carbon sequestration in soil.

The same can be argued for the CAT treatment in the third year that was further capable of significantly enhancing OC content in the largest macroaggregate size fraction and firmly sequester carbon, as shown by the OC fractionation losses which were lower than for No-CAT. Finally, the stable OC content for MIN and GMAN soils throughout the experimentation period and its significantly larger values than for TRA indicate that these soil management practices do also effectively sequestered carbon, though to a significantly lesser extent than COM and CAT treatments.

4.5 The Humified SOM Fraction and Its Characterization by Nuclear Magnetic Resonance Spectroscopy

The humified organic matter in soil, operationally separated in humic acid (HA), fulvic acid (FA), and humin (HU), is considered the most microbially stable reservoir of SOC, and the most important component for the maintenance of the soil physical–chemical and biological quality (Piccolo 1996). While the operationally non-extractable HU is the most passive fraction of humified matter (Hayes et al. 1989; Simpson et al. 2007), the extractable humic and fulvic fractions exert the essential role of intermediary compartments between the more labile organic compounds and the stable SOM pool. Humic matter results from the progressive accumulation in soil of hydrophobic organic molecules (Deport et al. 2006; Spaccini and Piccolo 2009) surviving the microbial degradation of plant residues. During humus formation, biolabile compounds present in the soil solution are progressively incorporated in the humic hydrophobic superstructures (Spaccini et al. 2000; Piccolo 2002). Moreover, humic molecules are also stabilized by the formation of complexes with different metals and adsorption on surfaces of soil minerals (Nebbioso and Piccolo 2009; Cornejo and Hermosin 1996).

The operational extraction of HS from soil consists in the alkaline separation of organic matter from soil mineral components and isolation of fulvic and humic acids based on their solubility at different pH values (Swift 1996). However, it is yet to be proven that the humic superstructures observed in extracts maintain the same intermolecular arrangement as in soil before extraction (Piccolo 1996; Spaccini et al. 2000; Kelleher and Simpson 2006; Nebbioso and Piccolo 2011). Nevertheless, due to the HS essential role in conferring soil quality and controlling all SOM transformation processes, their characterization is regarded as a valuable tool to appraise the effects of different management practices on long-term sequestration of organic carbon in cultivated soils (Swift 2001). Moreover, extractable humic

matter (HA and FA) was found to be in short-time dynamic interactions with other soil components (Spaccini et al. 2000). Thus, in this work, HS (jointly HA and FA) were alkaline extracted from soils subjected to the MESCOSAGR project treatments. The extracts were neutralized and purified as customary for humic acids (Spaccini et al. 2009), before being characterized by solid-state NMR spectroscopy.

The non-destructive solid-state ^{13}C cross-polarization-magic-angle-spinning (CPMAS) NMR technique provides the molecular distribution of organic carbons in solid matrices without extensive sample pre-treatment. This solid-state NMR technique is widely used to characterize the composition of litter, SOM, and humic substances, as well as the transformation of plant tissues in soil (Kögel-Knabner 2000; Hatcher et al. 2001; Conte et al. 2004; Zhou et al. 2010). Although CPMAS spectra are not strictly quantitative, a reproducible quantitation of molecular distribution may be achieved from spectra of soil humic fractions when acquisition parameters are correctly adopted (Kinchesh et al. 1995; Piccolo et al. 2005b; Spaccini et al. 2006).

A detailed comparison among NMR spectra may become excessively tedious and time consuming for the large number of CPMAS spectra obtained for HS extracts within the MESCOSAGR project. This burden can be reduced by applying chemometric methods or multivariate analyses. Among these, principal component analysis (PCA) is widely used to simplify interpretation of chemical and spectroscopic data for complex systems (Einax et al. 1997). The main purpose of PCA is to reduce the original data set, represented by an ' n ' dimensional space (where n is the number of variables or experimental results), into a few principal components (PC), which concomitantly retain the maximum percentage of original information contained in the data set. The principal components are derived as a linear combination of the original variables, such as NMR spectral areas. The variables are multiplied by loadings, which are vectors of constants generated during PCA. The numerical values of loadings reflect the importance of original variables in the direction of each PC. The resulting PC can be used to project the originally multidimensional data into only a two- or three-dimensional space, which is called a score plot (Brereton 2003). Even without a specific knowledge of statistical implications, these plots enable a rapid and direct evaluation of similarities, differences, and groupings among the original samples, which were extracted by PC analysis. Principal component analysis performed on ^{13}C NMR data has been successfully applied for statistical differentiation of humic substances and organic matter in agricultural soils (Novotny et al. 2007; Šmejkalova et al. 2008). Previous works have proved that PCA of ^{13}C -CPMAS NMR spectra of humic matter provide the required discrimination among heterogeneous samples and may be useful to evaluate SOM quality.

In this study, HS were extracted for each experimental site from the initial undisturbed soil and from soils at the end of each cropping cycle after harvesting of either maize or wheat. The four soil replicates from field treatments were mixed in order to obtain about 1 kg of composite soil sample. The free-lipid components were first removed from soil with two consecutive extractions with a 1:10 w/v of

dichloromethane–methanol (2:1 v/v) solution. The, humic substances were then extracted by shaking the soil (20 g) overnight with 100 ml of 0.1 M NaOH–Na₄P₂O₇ (1:1 v/v) solution under N₂ atmosphere. After centrifugation, the solid residue was washed with distilled water until pH 7. The supernatant and washings were combined, filtered on a quartz filter (Whatman GF/C), neutralized to pH 6 with 1 M HCl, dialyzed against deionized water, and freeze dried. The extractions were conducted in duplicate.

¹³C-CPMAS-NMR spectra of the compost materials as well as of HS extracted from soils were acquired on a Bruker AV-300, equipped with a 4 mm wide-bore MAS probe. Spectra were obtained with the following parameters: 13,000 Hz of rotor spin rate; 1 s of recycle time; 1 ms of contact time; 20 ms of acquisition time; 4,000 scans. Samples were packed in 4 mm zirconia rotors with Kel-F caps. The pulse sequence was applied with a 1H ramp to account for non-homogeneity of the Hartmann–Hahn condition at high spin rotor rates. For the interpretation of ¹³C-CPMAS-NMR spectra, the overall chemical shift range was divided into the following main resonance regions (Spaccini et al. 2009): alkyl-C (0–45 ppm); methoxyl-C and N-alkyl-C (45–60 ppm); O-alkyl-C (60–110 ppm); unsubstituted and alkyl-substituted aromatic-C (110–145 ppm); oxygen substituted aromatic-C (145–160 ppm); carboxyl- and carbonyl-C (160–200 ppm). The area of each spectral region (R_i^{abs}) was divided by the sum of all spectral areas, in order to obtain a relative percentage ($R_i^{\%}$):

$$R_i^{\%} = \left(\frac{R_i^{\text{abs}}}{\sum_i R_i^{\text{abs}}} \right) \times 100$$

The $R_i^{\%}$ values were used as variables for the multivariate statistical analysis.

In order to summarize the modifications brought about by different soil treatments on the molecular composition of humic extra cts, the following structural indexes were calculated from the relative amount of C distribution over the NMR spectra:

$$\text{HB} = \frac{\Sigma(0 - 45\text{ppm}) + (45 - 60\text{ppm}) + (110 - 160\text{ppm})}{\Sigma(45 - 60 \text{ ppm}) + (60 - 110 \text{ ppm}) + (160 - 190 \text{ ppm})}$$

$$\text{Lignin Ratio} = \frac{(45 - 60\text{ppm})}{(145 - 160 \text{ ppm})} \quad \text{AR} = \frac{(110 - 160\text{ppm})}{(0 - 190\text{ppm})}$$

The hydrophobic index (HB) is the ratio of signal intensity in chemical shift intervals for hydrophobic C components over that in intervals for hydrophilic C components. The Lignin Ratio is the ratio of signal intensity in the 60–45 ppm interval over that in the 160–145 ppm interval. The aromaticity index, Ar, is the relative percentage of aromatic components in the samples. The larger the HB and Ar values of a OM sample, the greater is its hydrophobic character and its content of aromatic molecules, respectively (Zhou et al. 2010). Likewise, the smaller the value

of the Lignin Ratio, the larger is the content of lignin-derived material in the humic extract or the lesser is the amount of biolabile hydrophilic carbon, such as in peptides (C–N in the 60–45 ppm interval) (Spaccini et al. 2009).

4.5.1 *NMR Spectra of Initial Soils and Composts Before Amendments*

The ^{13}C -CPMAS-NMR spectra of compost used for field treatments in the three experimental sites are shown in Fig. 4.1, whereas the relative distribution of signal areas is reported in Table 4.9. Since compost samples had a common origin, a similar C distribution was found in NMR spectra.

The spectra of mature composts were dominated by the alkyl-C (0–45 ppm) and O-alkyl-C (60–110 ppm) signals. The alkyl-C region comprised a prominent peak at around 30 ppm, mostly due to methylene carbon chains $(\text{CH}_2)_n$ and terminal methyl groups in plant lipid compounds, such as waxes and aliphatic biopolyesters.

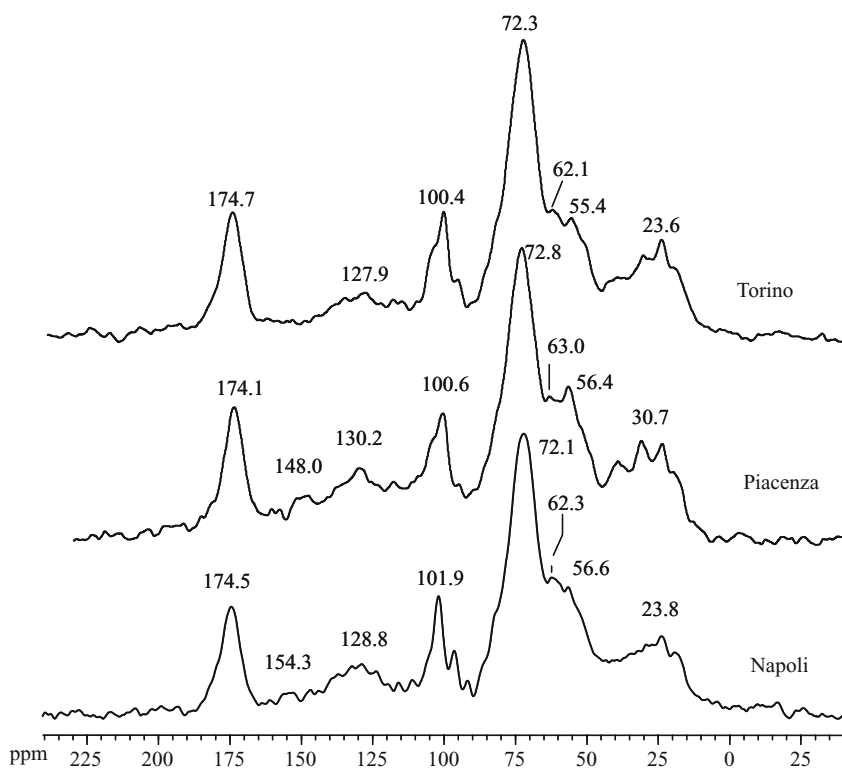


Fig. 4.1 ^{13}C -CPMAS-NMR spectra of the mature compost materials used for soil treatments at Torino, Piacenza, and Napoli

Table 4.9 Relative distribution (%) of signal area over chemical shift regions (ppm) in ^{13}C -CPMAS-NMR spectra of compost samples used for field treatments

Field site	Chemical shift regions						LR ^a	HB ^b
	Carboxylic-C (190–160)	Phenolic-C (160–145)	Aromatic-C (145–110)	O-alkyl-C (110–60)	CH ₃ O/C-N (60–45)	Alkyl-C (45–0)		
Torino	8.2	3.2	12.2	37.1	10.5	28.7	3.3	0.97
Piacenza	7.3	4.2	11.8	39.4	10.6	26.8	2.5	0.93
Napoli	8.7	4.1	11.6	36.9	10.5	28.3	2.6	0.97

^aLignin Ratio (LR) = (45–60 ppm)/(145–160 ppm)

^bHydrophobic index (HB) = $\Sigma[(0-45) + (45-60) + (110-160)]/\Sigma [(45-60) + (60-110) - (160-190)]$

The O-alkyl-C resonances are assigned to monomeric units in oligo and polysaccharidic chains of plant woody tissues (Vane et al. 2001; Wikberg and Maunu 2004). The intense signal around 72 ppm corresponds to the overlapping resonances of C2, C3, and C5 carbons in the pyranoside structure of cellulose and hemicellulose, whereas the signals at 105 ppm (sharp), 64 ppm, and 82–88 ppm (shoulders) are assigned to the anomeric C1, and C6 and C4 carbons, respectively, the latter being split in the presence of both amorphous and crystalline forms of cellulose (Atalla and VanderHart 1999). Plant woody tissues were also indicated by the 56 ppm shoulder of methoxy groups on the aromatic rings of guaiacyl and siringyl units in lignin structures (Liitiä et al. 2002; Zhou et al. 2010). The aromatic region (110–160 ppm) also revealed distinct resonances for O-substituted aromatic carbons in the 147–152 ppm interval, the 128 ppm broad signal for C-substituted or C-unsubstituted aromatic rings of lignin monomers (Albrecht et al. 2008), or cinnamic and ferulic units in suberin biopolymer (Stark et al. 2000; Graça and Santos 2007). Finally the sharp signal at 172 ppm is currently assigned to quaternary carbons of carboxyl groups. The OC composition shown by NMR spectra of compost closely resembles the molecular characteristics found for stable humified organic material in SOM (Spaccini and Piccolo 2007; Caricasole et al. 2011) with a large HB index (Table 4.9) due to a great content of hydrophobic molecules (Spaccini et al. 2008; Canellas et al. 2010).

Conversely, CPMAS-NMR spectra of HS extracted from initial control soils showed a predominance of hydrophilic carbon components and consequent low HB values (Fig. 4.2 and Table 4.10). These extracts revealed that 58–61% of total C was due to carbohydrates (110–60 ppm) and oxidized carboxylic groups (190–160 ppm), whereas less than 15–17% of carbon was in aromatic (160–110 ppm) and alkyl (45–0) structures (Table 4.10).

If humic extracts contained residual lignin carbon, the signal of methoxyl C at around 56 ppm and that of phenolic C in the 160–145 ppm region should bear a close relationship. An intense peak at 56 ppm was shown by NMR spectra of both compost samples and HS from control soils, but the intensity of the phenolic C resonance was different in these two organic materials (Figs. 4.1 and 4.2, respectively). A Lignin Ratio (see above) can be calculated using the relative areas of both 60–45 ppm and 160–145 ppm intervals in NMR spectra (Tables 4.9 and 4.10),

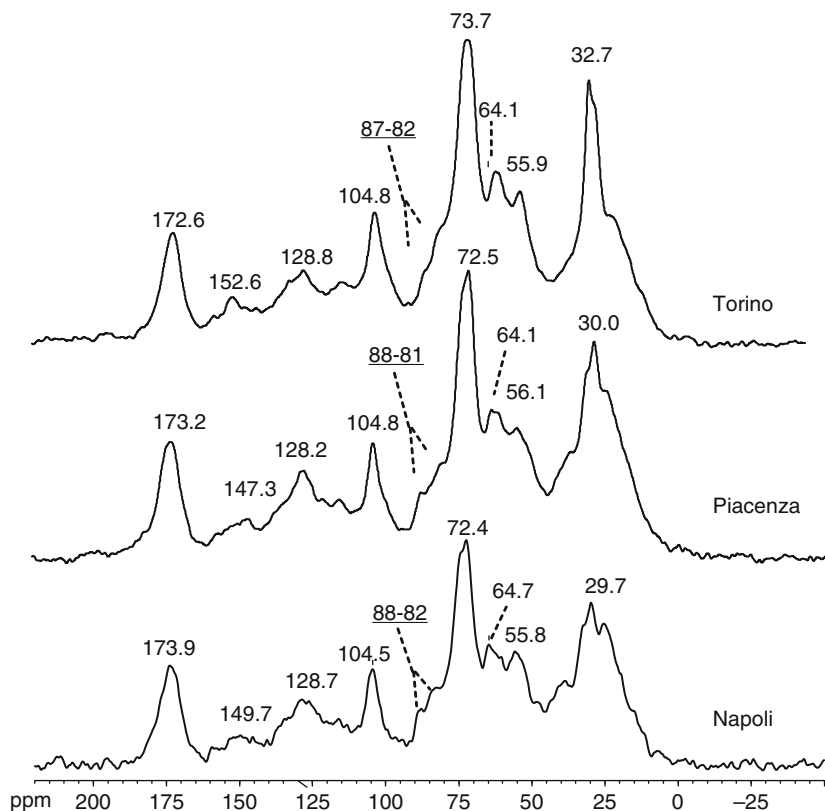


Fig. 4.2 ^{13}C -CPMAS-NMR spectra of humic substances extracted from initial control soils at Torino, Piacenza, and Napoli

Table 4.10 Relative distribution (%) of signal area over chemical shift regions (ppm) in ^{13}C -CPMAS-NMR spectra of humic substances from control soils

Field site	Chemical shift regions						LR ^a	HB ^b
	Carboxylic-C (190–160)	Phenolic-C (160–145)	Aromatic-C (145–110)	O-alkyl-C (110–60)	CH ₃ O/C-N (60–45)	Alkyl-C (45–0)		
Torino	9.9	2.5	12.1	48.3	12.6	14.6	5.0	0.55
Piacenza	11.0	1.7	9.0	51.2	9.6	17.5	5.6	0.49
Napoli	9.2	2.0	10.1	48.9	12.7	17.1	6.3	0.55

^aLignin Ratio (LR) = (45–60 ppm)/(145–160 ppm)

^bHydrophobic index (HB) = $\Sigma[(0-45) + (45-60) + (110-160)]/\Sigma[(45-60) + (60-110) - (160-190)]$

respectively). For compost samples, the Lignin Ratio varied from 2.5 to 3.3, while that for HS from control soils ranged between 5 and 6.3. These values thus indicate a likely content of lignified materials in compost samples (Vane et al. 2003), whereas this appears less probable for HS from control soil. In the latter, the intense

signal at 56 ppm should be better attributed to C nuclei in *alpha* position of amino-acid moieties (Quideau et al. 2001; Hayes et al. 2008) rather than to methoxyl C in Lignin.

In fact, NMR spectra of HS from control soils indicate that long-term cultivation without OM inputs reduced the amount of hydrophobic carbon, with consequent prevalent incorporation of biolabile components such as plant carbohydrates and peptidic derivatives. A small hydrophobicity of humic matter is a common feature in highly exploited and weathered soils (Piccolo et al. 2005b; Spaccini et al. 2006). The simultaneous increase of hydrophilic components with a decrease of alkyl-C in NMR spectra of SOM has been attributed to the presence of highly decomposable materials (Baldock et al. 1997; Webster et al. 2001) with short residence time, fast turnover, and slow accumulation rate.

4.5.2 Principal Component Analysis of NMR Spectra of Humic Substances Extracted from Soil Treatments

In order to compare the effect produced by soil treatments on the molecular composition of HS extracts at different experimental times, the areas of C signals in NMR spectra were integrated and their values elaborated with principal component analysis (PCA). The multivariate analysis for the first and third experimentation year at Torino, Piacenza, and Napoli sites of the MESCOSAGR project is shown in Figs. 4.3–4.5, respectively. The relative values (%) associated with the *x* and *y* axes in PCA diagrams stand for the total variation of NMR data explained by two statistical principal components (PC1 and PC2), while the dotted lines represent the loading vectors associated with the variables (ppm intervals) obtained from NMR spectra. Tables 4.11–4.13 show the variation of HS structural indexes throughout the three experimental years as calculated from NMR spectra for different soil treatments and experimental sites.

4.5.2.1 Torino Experimental Site

For the first and third experimentation years, PCA score plots of HS from TRA were compared with those from MIN and GMAN treatments (Fig. 4.3a). An overall treatment discrimination was given by PC1 between TRA and MIN and between TRA and GMAN. However, no consistent molecular variation was evident in HS composition among treatments for either replicates or experimental years. The loading vectors associated with signals at 110–60 ppm and 45–0 ppm indicate that HS extracted from MIN after the first year were rich in carbohydrates and poor in alkyl components, respectively. At the final year, an opposite distribution of carbohydrates and alkyl-C was revealed by the corresponding loading vectors in the TRA vs. MIN biplots. Moreover, although the principal components, PC1 and PC2,

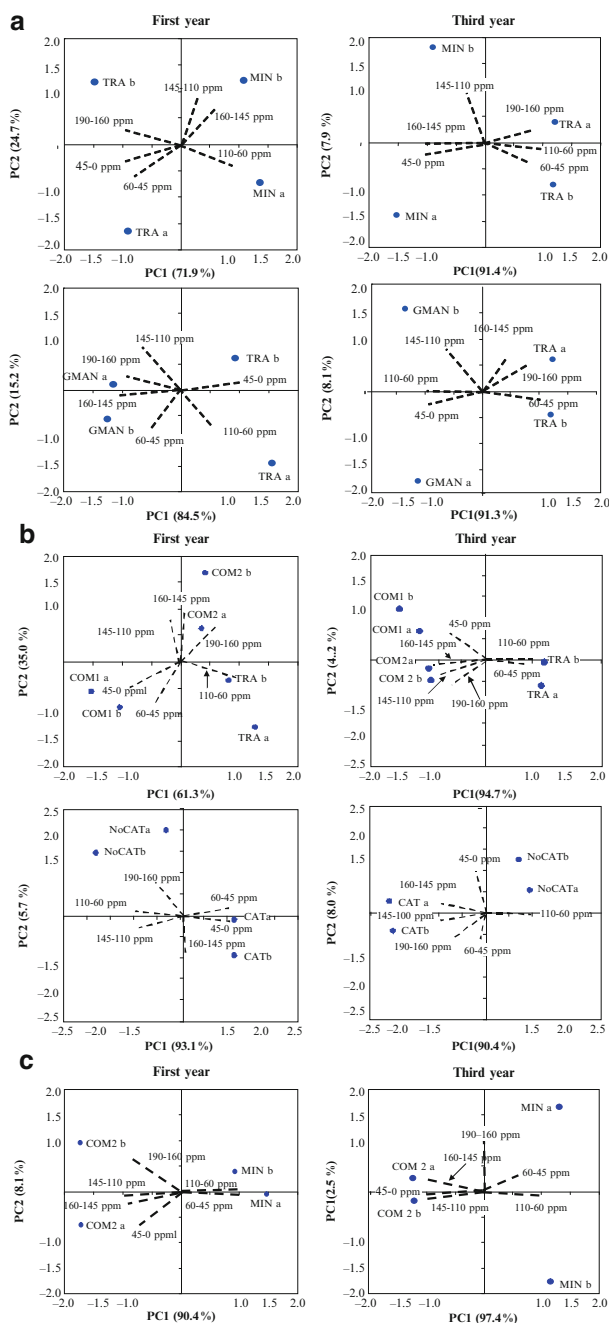


Fig. 4.3 Torino experimental site: biplots generated by principal component analysis of NMR spectra of humic substances extracted from soil treatments (a) TRA vs. MIN and TRA vs. GMAN both at first and third year of field experimentation (a and b indicate first and second replicates) (b) MIN vs. COM-1 and COM-2 and CAT vs. NoCAT, at first and third year of field experiments (small letters indicate replicates) (c) COM-2 vs. MIN at first and third year of field experiments (small letters indicate replicates)

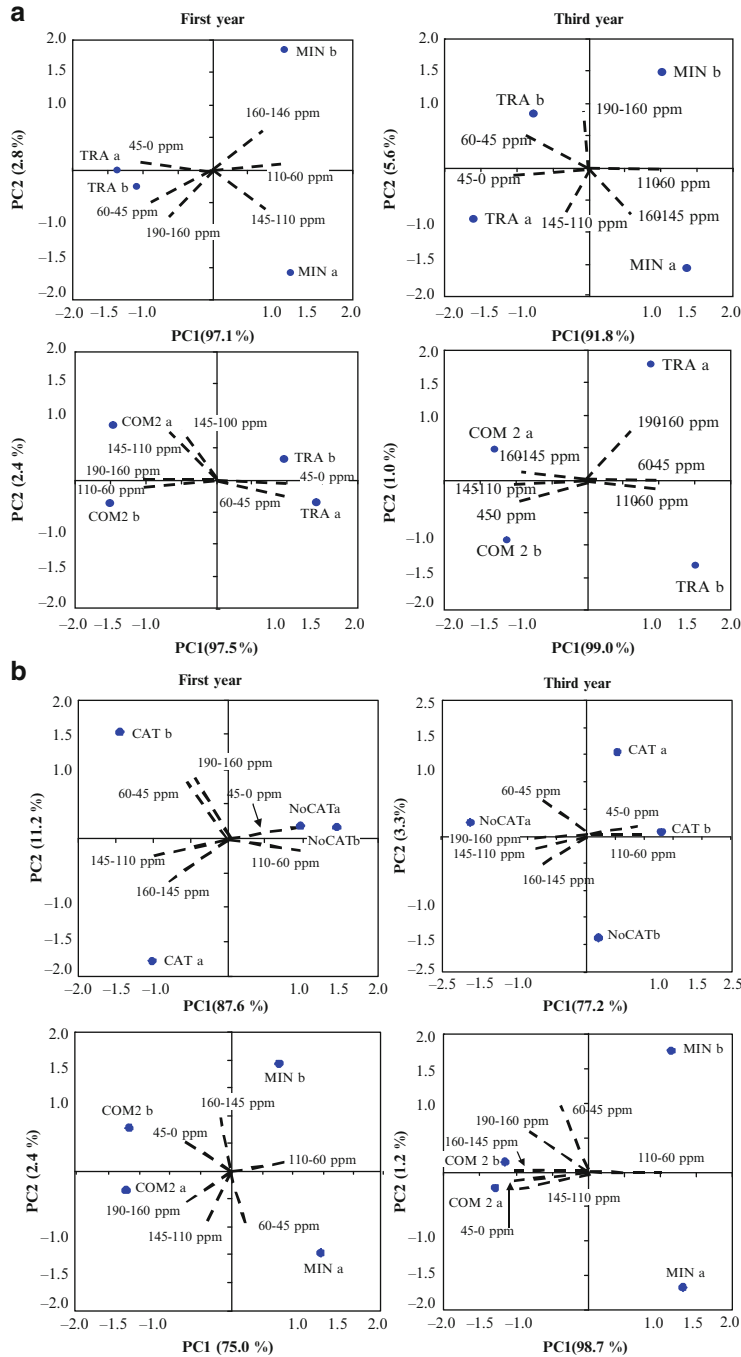


Fig. 4.4 Piacenza experimental site: biplot generated by principal component analysis of NMR spectra of humic substances extracted from soil treatments **(a)** TRA vs. MIN and COM-2 at first year of field experiments (*small letters* indicate replicates) **(b)** CAT vs. NoCAT and COM-2 vs. MIN at first and third year of field experiments (*small letters* indicate replicates)

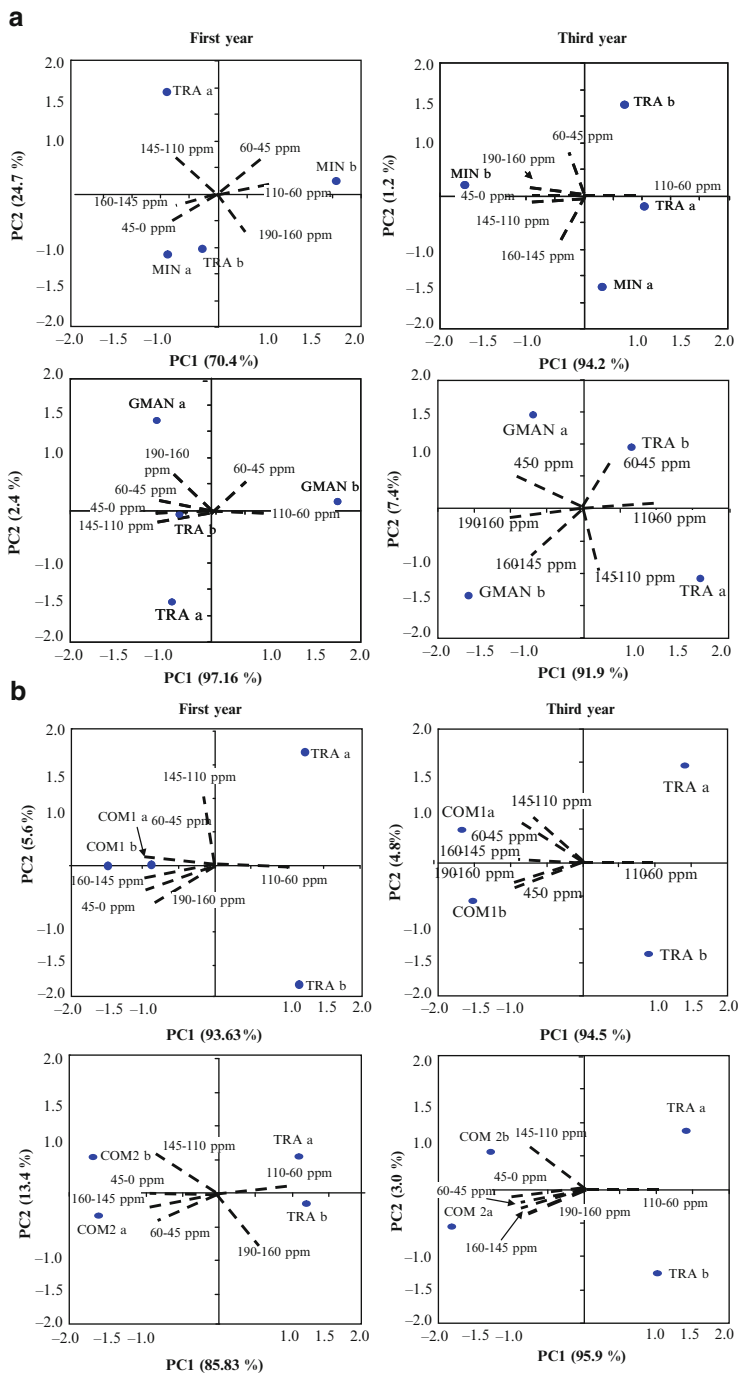


Fig. 4.5 (continued)

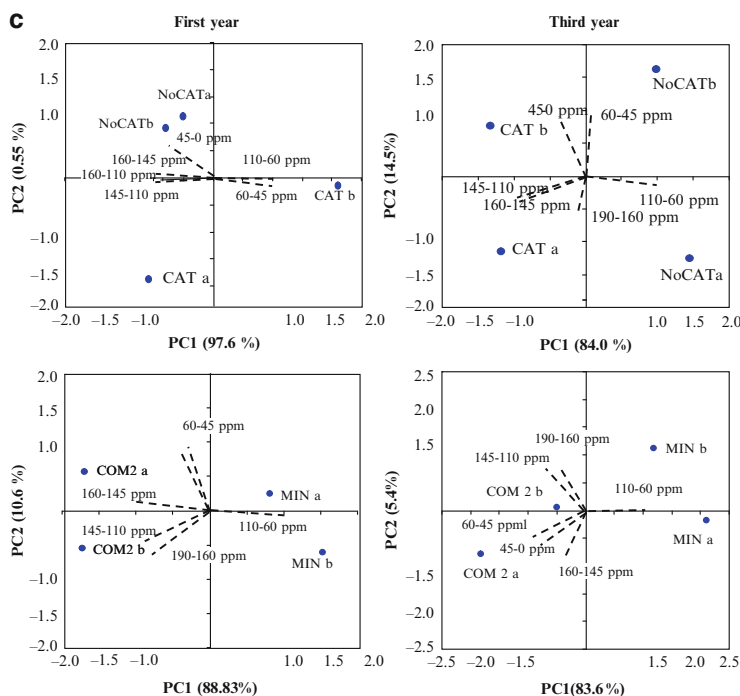


Fig. 4.5 Napoli experimental site: biplot generated by principal component analysis of NMR spectra of humic substances extracted from soil treatments (a) TRA vs. MIN and GMAN at first and third year of field experiments (*small letters* indicate replicates) (b) TRA vs. COM1 and COM2 at first and third year of field experiments (*small letters* indicate replicates) (c) CAT vs. noCAT and MIN vs. COM2 at first and third year of field experiments (*small letters* indicate replicates)

represented from 95 to 99% of total variability, no significant differences were found between HS from MIN and TRA in the loading vectors for molecular components.

A similar PCA was derived by comparing NMR spectra of HS extracted from TRA and GMAN (Fig. 4.3a). After 1 year, the main variation was along the PC1 component (84%) and mainly accountable to distribution of phenolic (160–145 ppm) and alkyl (45–0 ppm) compounds, whose amounts were, respectively, larger and lower in HS from GMAN than from TRA. After 3 years, the alkyl-C became more abundant in HS from GMAN, whereas no longer the distribution of phenolic and aromatic compounds represented a significant difference for HS from TRA. With progression of experimental time, NMR spectra of HS from both MIN and GMAN revealed a larger content of hydrophobic molecules than for HS from TRA (Table 4.11). In fact, both the slight HB increase and the more pronounced decrease of Lignin Ratio suggested a progressive incorporation of alkyl and phenolic compounds in HS from MIN and GMAN as compared to those from TRA.

Table 4.11 Torino experimental site, variation with experimental time of structural indexes HB, Lignin Ratio and Aromaticity (%), calculated from ^{13}C -CPMAS-NMR spectra of humic substances extracted from soil treatments for 3 years of experimentation

Treatment	First year		Second year		Third year	
	HB	Lignin Ratio	HB	Lignin Ratio	HB	Lignin Ratio
Maize						
TRA	0.73 (0.02)	4.61 (0.22)	0.76 (0.04)	3.92 (0.35)	0.69 (0.01)	4.19 (0.34)
MIN	0.71 (0.01)	3.95 (0.57)	0.79 (0.01)	3.17 (0.05)	0.83 (0.01)	3.16 (0.19)
GMAN	0.73 (0.01)	3.28 (0.02)	0.75 (0.03)	3.34 (0.44)	0.85 (0.02)	3.40 (0.06)
COM1	0.82 (0.02)	4.70 (0.29)	0.84 (0.03)	2.94 (0.10)	0.91 (0.01)	2.66 (0.39)
COM2	0.82 (0.02)	2.93 (0.03)	1.03 (0.13)	2.66 (0.24)	1.09 (0.01)	2.37 (0.05)
Treatment	First year		Second year		Third year	
	HB	Aromaticity	HB	Aromaticity	HB	Aromaticity
Wheat						
CAT	0.71 (0.01)	12.8 (0.22)	0.76 (0.04)	16.5 (1.01)	0.85 (0.04)	17.5 (0.37)
No-CAT	0.65 (0.01)	13.4 (0.56)	0.76 (0.04)	15.8 (0.49)	0.77 (0.02)	14.6 (0.14)

Number in parentheses indicate standard deviation ($n = 2$)

Hydrophobic index (HB) = $\Sigma[(0-45) + (45-60) + (110-160)]/\Sigma[(45-60) + (60-110) - (160-190)]$

Lignin Ratio = $(45-60)/(145-160)$

Aromaticity = $(110-160)/(0-190)$

Table 4.12 Piacenza experimental site, variation with experimental time of structural indexes HB, Lignin Ratio and Aromaticity (%), calculated from ^{13}C -CPMAS-NMR data of humic substances extracted from soil treatments for 3 years of experimentation

Treatment	First year		Second year		Third year	
	HB	Lignin Ratio	HB	Lignin Ratio	HB	Lignin Ratio
Maize						
TRA	0.63 (0.01)	6.46 (0.48)	0.74 (0.01)	3.52 (0.27)	0.73 (0.01)	3.61 (0.27)
MIN	0.60 (0.01)	5.86 (0.30)	0.78 (0.01)	3.45 (0.67)	0.66 (0.09)	3.95 (0.25)
COM2	0.71 (0.02)	5.58 (0.15)	0.98 (0.02)	2.36 (0.18)	0.93 (0.02)	2.01 (0.07)
Treatment	First year		Second year		Third year	
	HB	Aromaticity	HB	Aromaticity	HB	Aromaticity
Wheat						
CAT	0.67 (0.01)	16.2 (0.32)	0.74 (0.02)	14.2 (0.07)	0.70 (0.03)	14.3 (0.25)
No-CAT	0.64 (0.01)	10.9 (1.29)	0.76 (0.07)	15.2 (1.43)	0.73 (0.01)	14.1 (0.95)

Number in parentheses indicate standard deviation ($n = 2$)

Hydrophobic index (HB) = $\Sigma[(0-45) + (45-60) + (110-160)]/\Sigma[(45-60) + (60-110) - (160-190)]$

Lignin Ratio = $(45-60)/(145-160)$

Aromaticity = $(110-160)/(0-190)$

The PCA of NMR data for HS extracted from compost-treated plots revealed, with experimental time, an increased incorporation of exogenous hydrophobic organic carbon. After the first year, a significant discrimination ($\text{PC1} + \text{PC2} > 96\%$), was evident between both compost treatments and TRA (Fig. 4.3b). In fact, the loading

Table 4.13 Napoli experimental site, variation with experimental time of structural indexes HB, Lignin Ratio and Ar, calculated from ¹³C-CPMAS-NMR data of humic substances extracted from soil treatments for 3 years of experimentation

Treatment	First year		Second year		Third year	
	HB	Lignin Ratio	HB	Lignin Ratio	HB	Lignin Ratio
Maize						
TRA	0.80 (0.01)	2.67 (0.12)	0.72 (0.04)	4.07 (0.38)	0.85 (0.01)	3.03 (0.34)
MIN	0.94 (0.13)	2.23 (0.29)	0.85 (0.03)	2.50 (0.02)	0.83 (0.06)	2.85 (0.27)
GMAN	0.92 (0.08)	2.43 (0.32)	0.74 (0.05)	2.67 (0.00)	0.71 (0.05)	3.52 (0.09)
COM1	0.96 (0.06)	2.10 (0.11)	0.89 (0.04)	2.59 (0.15)	0.98 (0.01)	2.28 (0.12)
COM2	1.08 (0.02)	2.31 (0.23)	0.95 (0.03)	2.20 (0.20)	1.06 (0.03)	1.64 (0.13)
Treatment	First year		Second year		Third year	
	HB	Aromaticity	HB	Aromaticity	HB	Aromaticity
Wheat						
CAT	1.07 (0.04)	28.1 (0.16)	0.90 (0.07)	24.8 (1.71)	0.90 (0.00)	25.2 (0.66)
No-CAT	0.91 (0.03)	28.1 (0.84)	0.79 (0.02)	22.9 (0.81)	0.90 (0.04)	25.0 (0.71)

Number in parentheses indicate standard deviation ($n = 2$)

Hydrophobic index (HB) = $\Sigma[(0-45) + (45-60) + (110-160)]/\Sigma[(45-60) + (60-110) - (160-190)]$

Lignin Ratio = $(45-60 \text{ ppm})/(145-160 \text{ ppm})$

Aromaticity = $(110-160)/(0-190 \text{ ppm})$

vectors in the score plot indicated a larger incorporation of alkyl compounds (45–0 ppm) for COM-1 and of both phenolic (160–145 ppm) and aromatic components (145–110 ppm) for COM-2. The NMR spectra of HS from TRA were instead significantly dominated by O-alkyl-C units from carbohydrates and polysaccharides (110–60 ppm). The acquired hydrophobicity of compost-treated soils was also shown by the increased HB index (0.83) for NMR spectra of their HS extracts, as compared to those from TRA, whereas the low values for Lignin Ratio in HS from COM-2 suggested a large incorporation of lignin-like aromatic components (Table 4.11).

The increased hydrophobicity of HS from COM-1 and COM-2 throughout the experimental period shows that compost stabilized material has been progressively incorporated in soil humic fractions (Fig. 4.3b and Table 4.11). After the third year, the PCA of NMR data revealed enhancement of phenolic (160–145 ppm), unsubstituted aromatic (145–110), and alkyl C components (45–0 ppm) in HS from both COM treatments, while the loading vectors associated with NMR signals of hydrophilic C at 160–110 and 60–45 ppm were prevalent in HS from TRA. Furthermore, the large content of C–N bonds from peptidic moieties (60–45 ppm) in HS from TRA was accompanied by the increased Lignin Ratio values as a sign of progressive reduction of hydrophobicity in soil humus from TRA. The contribution of composted matter (mainly alkyl and lignin derivatives) in the molecular composition of stable soil HS was shown by the NMR-derived HB index and Lignin Ratio for COM-1 and COM-2 soil treatments. The enhanced hydrophobicity of HS from COM-2 was also evident in comparison with NMR results obtained for MIN (Fig. 4.3b). The loading vectors associated with alkyl (45–0 ppm) and phenolic

and aromatic (160–110 ppm) C regions, for both the first (PC1 90.7%) and third experimental years (PC1 97%) indicated the occurred incorporation of hydrophobic materials in HS from COM-2.

After the first experimental year, only minor differences were found between HS from soil treated with the biomimetic catalyst (CAT) and those from its control (No-CAT) (Fig. 4.3c). Conversely, after 3 years of treatment, HS from CAT revealed a preferential distribution along the PC1 (90.4%) of signals in the 160–145 and 145–110 ppm regions, thereby showing an increased incorporation of phenolic and aromatic C in the soil humic fraction. Such enhanced content of aromatic components in HS from CAT was consistently accompanied by progressive increase of the NMR aromatic index, as compared to that of No-CAT (Table 4.11).

4.5.2.2 Piacenza Experimental Site

No distinct treatment effects on soil humic composition were revealed by the loading vectors along both PC1 and PC2 in the biplots from NMR of HS extracted from either TRA or MIN (Fig. 4.4a). After the first year, the low values for HB and the large ones for Lignin Ratio for HS from both TRA and MIN indicated the predominance of hydrophilic compounds, such as carbohydrates and peptidic moieties. However, at the experiments end, an incorporation of lignified plant material was revealed by the slight decrease of Lignin Ratio for both soil treatments (Table 4.12).

The low hydrophobic character in HS from TRA and MIN was evident when compared with that resulting from NMR data of HS from COM-2 (Fig. 4.4a, b). In fact, for both sampling times, NMR data provided a positive discrimination along PC1 for loading vectors associated with alkyl (45–0 ppm) and overall aromatic (160–145 and 145–110 ppm) carbons in HS from COM-2. Conversely, the HS from COM-2 were negatively correlated with loading vectors associated with carbohydrates signals (110–60 ppm). Table 4.12 supported the occurred incorporation of hydrophobic molecules from alkyl components and lignin derivatives of compost in HS of COM-2. The molecular distribution, in fact, indicates an increase of HB index and a concomitant progressive decrease of Lignin Ratio throughout the experimental period.

After 1 year of soil treatment with biomimetic catalyst, an increase of aromatic C in HS extracts was suggested by the distribution of phenolic (160–145 ppm) and aromatic (145–100 ppm) C signals along biplot PC1 (87%) (Fig. 4.4b) and by the larger Ar index value (16.2%) with respect to that (10.9%) of control (No CAT) (Table 4.12). However, CAT treatment apparently lost effectiveness later in the experimentation, as revealed by the Ar index of HS from CAT, whose value approached that of control, and by the PCA loading vectors which no longer discriminated between CAT and No-CAT (Fig. 4.4b).

4.5.2.3 Napoli Experimental Site

As in the case of Torino and Piacenza, also for Napoli field experiments, few differences were found by PCA among NMR results for HS from TRA, MIN, and GMAN (Fig. 4.5a). In fact, although from 94 to 99% of total variability for the OC distribution was covered by PCA, at every sampling time, no specific distinction was appreciated for the loading vectors associated with different NMR chemical shift regions in HS from TRA, MIN and GMAN.

However, the latter treatments did induce a significant incorporation of hydrophobic alkyl and lignin compounds in HS, if their values for HB and Lignin Ratio (Table 4.13) were compared to those of the initial soil before the experimentation (Table 4.10). This significant difference may have been due to both the low OC content in the initial undisturbed soil and the large surface area offered by the clay content in this soil. These two conditions may have enhanced the physical—chemical affinity of organic components to soil particles (Staunton and Quiquampoix 1994; van Oss and Giese 1995) and favored the stabilization of the organic molecules that entered the subsoil during cropping seasons (Webster et al. 2001; Piccolo et al. 2004; Winkler et al. 2005).

Hydrophobic molecules from compost were incorporated more in HS from COM treatments than for TRA, as suggested by the PCA of NMR results (Fig. 4.5b). After the first year, this incorporation was already shown by the loading vectors of both aliphatic (45–0 ppm) and phenolic (160–145 ppm) components in HS from both COM-1 and COM-2, though only that associated to alkyl carbon had >95% of statistical significance. Conversely, the loading vector for hydrophilic carbohydrate carbon (110–60 ppm) characterized HS from TRA. After 3 years, the distribution of loading vectors associated to phenolic (160–145 ppm), aromatic (145–110 ppm), and alkyl (45–0 ppm) carbons confirmed the larger hydrophobicity of HS from compost-treated soils, while a persistent content of hydrophilic compounds (110–60 ppm) was found for TRA. The incorporation of alkyl hydrophobic molecules in compost-amended soil was again evident by comparing the OC distribution in HS from COM-2 and MIN (Fig. 4.5c). In fact, the PC1 discrimination represented 88 and 83% of total variation for initial and final experimental time, respectively, showing a positive correlation of hydrophilic O-alkyl-C in HS from MIN, and of alkyl components in HS from COM-2.

The progressive decrease of HB index in HS from MIN and GMAN and the simultaneous increase of Lignin Ratio (Table 4.13) revealed that reduced tillage or green manuring promoted the progressive loss of stable hydrophobic OM from soil (Piccolo 1996; Spaccini et al. 2006). Conversely, HB index and Lignin Ratio for COM treatments indicated incorporation of hydrophobic organic molecules from compost into soil humic substances (Table 4.13). Moreover, the Lignin Ratio in HS from COM-1 (2.28) and COM-2 (1.64) was lower than that of the very compost used for soil treatments (2.5 in Table. 4.9). This suggests that there must have been an incorporation and sequestration into SOM of additional lignin components derived from crop residues left on soils (Spaccini et al. 2000).

After 1 year of experimentation, the PCA of NMR molecular distribution in HS from CAT and that from No-CAT was not significantly different (Fig. 4.5c). This supports the Aromaticity index that indicated a similar content of total aromatic components for both treatments (Table 4.13). However, a significant increase in HB index was noted for CAT for both the first and second experimentation years (Table 4.13). After 3 years, a positive correlation was found for HS from CAT with the loading vector of unsubstituted and alkyl-substituted aromatic components (145–110 ppm) along PC1 (84% of total variation), though no differences were detected in HS total aromatic content between CAT and No-CAT (Table 4.13).

4.6 Concluding Notes on Soil Organic Carbon

The field experiments of the MESCOSAGR project have provided sound indications that the innovative soil treatments, such as amendment with mature hydrophobic compost and in situ SOC photo-polymerization through biomimetic catalysis, sequestered carbon and stabilized SOM more than conventional soil management practices. In fact, the variability in SOC content throughout the experimentation period shown by both MIN and GMAN suggests that these treatments were not able to persistently stabilize OC in both bulk samples and soil particle sizes more than TRA. Moreover, NMR evaluation of soil HS extracted from MIN and GMAN excluded any significant and persistent variation in SOM chemical quality with respect to TRA.

Conversely, results obtained for COM-1, COM-2, and CAT plots for all experimental sites during the whole experimentation, suggest an overall positive effect of soil treatments with both humified compost and biomimetic catalyst on SOC accumulation and stabilization.

Notwithstanding the initial decrease of OC content due to a priming effect observed in the first year after compost addition to soils of Napoli and Torino, compost treatments progressively increased stable OC in bulk soils and soil aggregates in subsequent years. Such a persistent incorporation of hydrophobic components in compost-treated soils was also indicated by the molecular characteristics of HS extracted from different sites throughout the experimentation.

Soil treatment with biomimetic catalyst was found to positively affect both total SOC content and HS molecular characteristics. However, its effect was maintained throughout the whole experimental period only for Piacenza, whereas a decreasing effect was noted for Torino and Napoli in the third year. These contrasting results may be related to the different soil textural composition in the three experimental sites. The Torino sandy-loam soil and the still highly sandy Napoli soil may have not sufficiently adsorbed the water-soluble iron–porphyrin catalyst on soil particles, and it was partially lost by leaching. Conversely, an effective adsorption of biomimetic catalyst on mineral surfaces may have occurred in the heavy textured silty-clay soil of Piacenza, thereby allowing the catalyst to exert a prolonged photo-polymerization activity. However, the effectiveness of the catalyst when in close

interaction with fine mineral particles in all three experimental sites was confirmed by the increased OC distribution in small soil aggregate sizes. In fact, as compared to No-CAT, absolute and relative OC content in CAT soils revealed a significant greater OC incorporation in microaggregates and smallest macroaggregates.

In the quest of summarizing the practical results of our field studies, Tables 4.14–4.16 show the ton ha^{-1} of TOC content in bulk soil for the various treatments in the three experimental sites. The values for MIN and GMAN confirm the short-term and even negative effect of both treatments on SOC accumulation.

Table 4.14 Torino experimental site, total organic carbon (ton ha^{-1}) in bulk soil under different treatments for three experimentation years

Treatments	First year		Second year			Third year		
	TOC ^a	Δc^b	TOC	Δc	Δr^b	TOC	Δc	Δr
Maize								
Control	60.4							
TRA	60.9	0.5	60.4	–	–	60.9	–	–
MIN	56.2	–4.2	60.4	0.5	4.7	59.3	–2.1	–1.6
GMAN	62.0	1.6	56.7	–3.1	–4.7	63.0	1.6	5.8
COM-1	62.0	1.6	63.5	3.7	2.1	66.7	5.3	2.6
COM-2	59.9	–0.5	65.1	5.2	5.8	69.3	7.9	3.7
Wheat								
No-CAT	65.1	–	62.0	–	–	71.9	–	–
CAT	69.3	4.2	68.3	9.4	2.1	72.5	–9.4	–8.9

^aTotal organic carbon = $\text{OC (g kg}^{-1}) \times \text{bulk density (1.5)} \times 10,000 (\text{m}^2) \times \text{plow depth (0.35 m)}$

^b Δ = yearly difference in TOC for each treatment in respect to TRA (or No-CAT) and to either initial or previous year TOC. E.g.: Δc (cumulative) for MIN 2nd year = (MIN 2nd year–TRA 2nd year) – (TRA 2nd year–TRA 1st year); Δr (relative) for MIN 2nd year = (MIN 2nd year–MIN 1st year) – (TRA 2nd year–TRA 1st year)

Table 4.15 Piacenza experimental site, total organic carbon (ton ha^{-1}) in bulk soil under different treatments for three experimentation years

Treatments	First year		Second year			Third year		
	TOC ^a	Δc^b	TOC	Δc	Δr^b	TOC	Δc	Δr
Maize								
Control	78.3							
TRA	76.0	–2.3	75.5			85.1		
MIN	66.0	–12.3	74.2	–0.9	8.6	101.9	16.8	17.7
COM-2	68.7	–9.6	77.4	2.3	9.1	106.9	21.8	19.6
Wheat								
No-CAT	74.2		55.5			77.4		
CAT	76.0	1.8	61.0	24.1	3.6	88.3	–10.9	5.5

^aTotal organic carbon = $\text{OC (g kg}^{-1}) \times \text{bulk density (1.5)} \times 10,000 (\text{m}^2) \times \text{plow depth (0.35 m)}$

^b Δ = yearly difference in TOC for each treatment in respect to TRA (or No-CAT) (Δc) and to either initial or previous year TOC (Δr). E.g.: Δc (cumulative) for MIN 2nd year = (MIN 2nd year–TRA 2nd year) – (TRA 2nd year–TRA 1st year); Δr (relative) for MIN 2nd year = (MIN 2nd year–MIN 1st year) – (TRA 2nd year–TRA 1st year)

Table 4.16 Napoli experimental site, total organic carbon (ton ha^{-1}) in bulk soil under different treatments for three experimentation years

Treatments	First year		Second year			Third year		
	TOC ^a	Δc^b	TOC	Δc	Δr^b	TOC	Δc	Δr
Maize								
Control	51.5							
TRA	43.6	-7.8	44.6	-	-	46.6	-	-
MIN	48.0	-3.4	50.5	4.9	1.5	49.5	1.0	-2.9
GMAN	51.5	0.0	49.0	3.4	-3.4	49.0	0.5	-2.0
COM-1	46.6	-4.9	49.0	3.4	1.5	51.5	2.9	0.5
COM-2	54.9	3.4	55.4	9.8	-0.5	61.3	12.7	3.9
Wheat								
No-CAT	53.9		49.0	-	-	55.9	-	-
CAT	51.0	-2.9	54.4	10.3	8.3	56.8	-5.9	-9.3

^aTotal organic carbon = OC (g kg^{-1}) $\times$ bulk density ($1.5 \times 10,000 \text{ (m}^2) \times$ plow depth (0.35 m)

^b Δ = yearly difference in TOC for each treatment in respect to TRA (or No-CAT) and to either initial or previous year TOC. E.g.: Δc (cumulative) for MIN 2nd year = (MIN 2nd year-TRA 2nd year) - (TRA 2nd year-TRA 1st year); Δr (relative) for MIN 2nd year = (MIN 2nd year-MIN 1st year) - (TRA 2nd year-TRA 1st year)

Table 4.17 Net total organic carbon (ton ha^{-1}) in bulk soil under different treatments for three experimentation years, as obtained by subtracting from measured SOC either 2.7 or 5.4 ton ha^{-1} of OC added with the COM-1 and COM-2 treatments, respectively

Treatments	First year	Second year		Third year	
	$n\Delta c^a$	$n\Delta c$	$n\Delta r^b$	$n\Delta c$	$n\Delta r$
Torino					
COM-1	-1.6	0.4	-1.1	3.1	0.4
COM-2	-6.5	-0.7	-0.2	3.0	-1.2
Piacenza					
COM-2	-12.7	-3.6	3.2	16.4	24.2
Napoli					
COM-1	0.2	1.7	-0.25	2.2	-0.2
COM-2	5.9	5.4	-4.9	9.3	0.5

^a $n\Delta c$ (net cumulative variation) = (TOC COM-1-TOC TRA) - 2.7; (TOC COM-2-TOC TRA) - 5.4

^b $n\Delta r$ (net relative variation) = (TOC COM-1_{2nd year}-TOC COM-1_{1st year}) - 2.7; (TOC COM-2_{2nd year}-TOC COM-2_{1st year}) - 5.4

On the contrary, both cumulative (Δc) and relative (Δr) increments of TOC for compost and catalyst treatments indicate their relevant potential in favoring the long term sequestration and stabilization of organic carbon in soil.

After 3 years, the hydrophobic protection mechanism implied in the compost amendments to soils provided an amount of fixed SOC ranging from 3 to 22 ton ha^{-1} more than TRA, depending on experimental site.

The potential of mature compost treatment to sequester OC in soil is further revealed by the net relative increase of TOC when calculated by subtracting from soil carbon the compost OC annually added to soils (Table 4.17). Except for an

initial SOC decrease at Torino and Piacenza, net SOC increments were generally observed. This shows that the hydrophobic protection mechanism exerted by compost progressively protected, against biotic and abiotic oxidation, both the indigenous SOM and the seasonal OC inputs (microbial biomass, plant roots, and crop residues). The net carbon sequestration obtained over the 3 years by hydrophobic protection of compost resulted thus in much greater than 0.5 ton C ha⁻¹ year⁻¹ achievable by reduced or zero tillage methods (Freibauer et al. 2004).

Though more variable, the OC fixed in the first and second years by the mechanism of in situ catalyzed SOM photopolymerization varied from 4 to 24 ton ha⁻¹. Also these values overcome by far the sequestration potential of simple tillage management practices.

Both innovative methods adopted in the MESCOSAGR project appear then liable to become important to turn agricultural soils into OC sinks and contribute to alleviate the greenhouse effect and the global changes.

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Chapter 5

The Stable Isotopes Approach to Study C and N Sequestration Processes in a Plant–Soil System

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Abstract This chapter reviews the main methods for tracing N and C stable isotopes in natural and agricultural systems following organic and mineral amendments to soil. Moreover, we present the results obtained from two field experiments conducted, within the MESCOSAGR project, to evaluate either the fate and flow rate of N added as ^{15}N -compost in a maize–soil system or the contribution of sorghum roots to soil organic carbon. Compost contribution to plant nutrition was about 20% of applied N in the first experimentation year, while this value decreased in the following 2 years. The mineralization rate in the first year was anyhow variable depending on compost maturity and composition, while compost amendments mostly affected the inclusion of ^{15}N in soil macro-aggregates. The compost-derived nitrogen sequestered in soil, due to repeated amendments, was estimated to account for 34.2, 38.2 and 42.5% of total N-compost for the first, second and third years, respectively. On the other hand, it was found that soil carbon derived from sorghum residues reached about 28% after 3 years, though this percentage decreased with depth, and more rapidly below 30 cm.

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5.1 Introduction

The use of organic and composted residues as a source of nutrients is increasingly important in sustainable management techniques. Compost has a great potential to recycle carbon and nutrients that otherwise would have to be disposed of, to improve the energy efficiency of the cropping systems, and to contribute to sequestration of soil C. Replacing mineral N fertilizers with compost could increase the environmental sustainability of fertilizers and the agro-ecosystem. However, the ability of compost to completely sustain crop N uptake through mineralization needs to be confirmed.

The quantification of nitrogen cycling in agriculture requires the use of special techniques, especially to measure the recovery of N fertilizers in soil. Stable isotopes have been used to trace the fate and movement of nutrients in the environment and to quantify the fractional contribution of soil organic matter (SOM) and added organic material to plant uptake. The calculation of N losses from crop–soil systems can successfully be done using ^{15}N -labelled fertilizer (Powelson et al. 1992). It has been shown that N uptake by crops from organic inputs such as plant residues or manures is often less than 20%. However, although this amount seems little, it has been widely accepted that organic inputs play a significant role in the long-term build-up of organic matter in soil and its stabilization. The use of ^{15}N in organic fertilizer studies has significantly advanced our understanding of N release from organic materials. ^{15}N direct labelling techniques are used to study crop residue or green manure contribution to plant nutrition. Green manures can be easily obtained by growing crops fertilized with ^{15}N tracer. The above- or belowground material is then harvested and added as residues to unlabelled soil where the next crop is grown. This crop is then harvested and the percentage nitrogen in the crop derived from added residues can be calculated (Hauck and Bremner 1976).

Indirect techniques have also been used to study plant N uptake from organic residues. ^{15}N tracer is added to soil, and treatments with and without residues (no-residue controls) are set up. The no-residue controls should have an ^{15}N enrichment that reflects the joint soil plus ^{15}N –N fertilizer pool, while the residue treatments should have a lower ^{15}N enrichment due to input of unlabelled N originating from the decomposing residue. The nitrogen derived from residue (*N_{dfr}*) can then be calculated. This is the same principle used in ^{15}N dilution methods to estimate the biological nitrogen fixation.

Measurements of natural ^{15}N abundance ($\delta^{15}\text{N}$) in organic materials and in soil particles can be used to characterize their origin and the rate of N changes involved. The variation in natural abundance as $^{15}\text{N}/^{14}\text{N}$ ratio has been used in fewer studies (e.g., Kerley and Jarvis 1997) with respect to artificially enriched materials. One reason is that it is quite difficult to identify well-defined natural N-pools of considerably different isotopic composition (Gerzabek et al. 2001). This is in contrast to C, where material from C_3 and C_4 plants can be used as tracers due to their significant differences in $^{13}\text{C}/^{12}\text{C}$ ratios. Variation in natural abundance of

N stable isotopes in soil is explained by both (1) the input of N from different sources with different isotopic compositions and (2) isotopic fractionations during transport and especially microbial turnover (Macko and Estep 1984). Fractionation in the soil N cycle complicates the quantification of N turnover and dynamics derived from typical agricultural N sources using natural abundance ($^{15}\text{N}/^{14}\text{N}$ ratio), although in some studies involving reference treatments the quantification of source-derived N was partly successful (e.g., Wagner 1991).

Studies on the decomposition and mineralization of labelled plant residues have commonly been conducted under well-defined greenhouse or laboratory conditions (Cortez and Hameed 2001; Semenov et al. 2001). In addition, various field studies have observed the decomposition of labelled organic matter in agricultural croplands. For example, Voroney et al. (1989) monitored field decomposition of labelled wheat straw in the short- (2 years) and long-term (up to 10 years) in the fields using bulk soil material without any soil fractionation.

Researchers used N isotopes to track nutrient partitioning among different soil constituents. Tiessen et al. (1984) showed that sand and silt fractions had a low enrichment, whereas clays had a high enrichment. They related this difference to an association of low enrichment plant material with larger particles. In addition, because clay provides N transformation sites and is physically associated with organic materials, there is an enrichment in this fraction.

Aita et al. (1997) and Haynes and Beare (1997) analysed the short-term (2 years) decomposition of labelled plant residues by separating soil into particle-size fractions. Within the first year of the study, the authors showed a rapid decrease of ^{15}N in coarse and light fractions that represent decomposing plant residue material. As a consequence, a concomitant ^{15}N enrichment in fine particle-size fractions ($<50\text{ }\mu\text{m}$) was observed, including microbial derived organic constituents (Haynes and Beare 1997). Swanston and Myrold (1997) showed that after 21 months of incubation only one-third of the ^{15}N recovered from labelled red alder leaves was found in the heavy fractions.

Gerzabek et al. (2001) studied the nitrogen distribution and ^{15}N natural abundance in particle size fractions in a long-term agricultural field experiment. They reported that in most cases $\delta^{15}\text{N}$ values increased with decreasing particle size. They also concluded that the natural abundance of ^{15}N in bulk soil and particle size fractions was significantly altered by the long-term application of Ca-nitrate and organic manures. Kölbl et al. (2006) analysed the short-term (570 days) decomposition of labelled mustard litter by separating soil into particle-size fractions. After 570 days of application of ^{15}N -mustard litter to an agricultural cropland, the distribution of ^{15}N was measured in particulate organic matter (POM) fractions and in fine mineral fractions (fine silt- and clay-sized fractions). After 570 days, only 2.5% of the initial ^{15}N amount was found in POM fractions, with larger amounts in aggregate-occluded POM than in free POM. After this period, stabilization of initial ^{15}N in fine silt- and clay-sized fractions amounted to 10% in high-yield soils, but 20% in low-yield soils, while 70–85% of the added ^{15}N was lost. They also found that in some croplands up to 25% of applied ^{15}N was stabilized in clay-sized fractions after 161 days.

Mueller et al. (2009) found that the POM fractions in 0–2 cm of the topmost soil layer showed decreasing ^{15}N concentrations in the free particles and increasing concentrations in the occluded particles. Swanston and Myrold (1997) also observed the highest ^{15}N recovery in <5 cm of the topmost soil layers and the light fraction, while below a 5-cm depth, the ^{15}N recoveries were low and variable. In a decomposition study using ^{15}N -labelled beech litter, Zeller et al. (2000) found that 62% of released N came from the surface soil after 3 years but only 12% came from a depth below 2 cm.

The annual input of organic materials to the soil from crops is an important component in the study of organic matter (Balesdent and Balabane 1992). Studying C sequestration, C budget and C dynamics entails quantifying the organic carbon (C) input derived from crop roots to cultivated soil (Hobbie et al. 2002).

Roots play a dominant role in C and N soil cycles (Gale et al. 2000a; Puget and Drinkwater 2001) and may have a relatively greater influence on soil organic C and N levels than the aboveground plant biomass (Boone 1994; Norby and Cotrufo 1998). Root-derived sources of soil organic C (SOC) are the materials released from roots during growth: mucilage, sloughed off root tips, root exudates, gradual loss of cells by the fully functional roots and decaying roots (van Noordwijk et al. 1994).

Amounts of root-derived C or root biomass vary according to environmental conditions, management systems, crop genotypes, and the physical, chemical and biological properties of the soil (Balesdent and Balabane 1992). Root development is also particularly sensitive to variations in the supply and distribution of inorganic nutrients and water (Steingrobe et al. 2001). More importantly, root mucilage, root exudates and detached root tips contribute considerably to total SOC input in the soil (Crawford et al. 1996), which is difficult to measure by conventional methods.

Helal and Sauerbeck (1987) estimated that the amount of C released from plants as rhizodeposition could be more than 580 kg C ha⁻¹. This amount increases microbial activity and influences N mineralization in the soil (Bakken 1990; Texier and Biles 1990). As much as 7–43% of the total above- and belowground plant biomass can be made up of roots (Kuo et al. 1997). Maize and winter cover crop roots can supply from 400 to 1,460 kg C ha⁻¹ during a growing season (Kuo et al. 1997). Balesdent and Balabane (1996) observed that maize (*Zea mays* L.) roots contributed 1.6 times more C to organic soil C than stover. Root-derived C is retained and forms more stable aggregates than shoot-derived C (Gale et al. 2000a, b). Liang et al. (2002) found that maize roots contributed as much as 12% of soil organic C, 31% of water soluble C and 52% of microbial biomass C within a growing season. The carbon contribution from maize root biomass and rhizodeposition to soil organic C can be as much as 1.7–3.5 times greater than that from stover (Wilts et al. 2004).

Fenández et al. (2003) studied carbon allocations in a sweet sorghum–soil system using ^{14}C as a tracer in order to assess the contribution of the crop to carbon storage in the soil. Sampling was performed 24 h after labelling and at harvest they concluded that 4–16% of ^{14}C present in the sorghum–soil was located in the soil fine fraction (<2 mm). At the harvest, the proportion of ^{14}C present in the soil

accounted for 7–9% of the ^{14}C presented in sorghum–soil system. The plant-derived soil carbon was estimated at $0.10\text{--}0.12\text{ g C plant}^{-1}\text{ day}^{-1}$, the carbon total amount captured by sweet sorghum was estimated at 1.44 kg C m^{-2} over the whole growth cycle: 0.82 kg C m^{-2} in the aboveground biomass, 0.52 kg C m^{-2} in the below ground biomass and 0.10 kg C m^{-2} in the soil carbon pool.

This chapter reviews some of the most useful methodologies and applications of N and C tracers to trace the fate of added organic or mineral material to the soil in natural and agricultural systems. Moreover, the main results from two field experiments are presented: one with N and the other with C isotopes, carried out within the framework of the MESCOSAGR project (see Foreword and Chap. 3). Both experiments were aimed at evaluating the fate of added compost to soil cropped with maize or sorghum. The first quantified the fate and flow rate of N in plant–soil systems, while the second monitored soil C sequestration associated with sorghum plant rhizodeposition.

5.2 Nitrogen Isotope Approach to Study N Dynamics

Nitrogen isotopes have been applied to trace the flows and fate of N since their discovery at the beginning of the twentieth century. The research study by Norman and Werkman (1943) exemplifies their first agronomic application (Hauck et al. 1994). Of all the different N isotopes, only the stable ^{15}N and ^{14}N , and the radioactive ^{13}N , have been used as tracers in research. While ^{15}N and ^{14}N have been used in routine measurements, radioactive ^{13}N have been restricted to specific environmental and basic biological studies. In addition to its radioactivity, thus requiring operator protection and safe disposal, the half-life of ^{13}N is very short and the isotope must be produced close to the experimental site. However, since its detection limit is much smaller than ^{15}N , the use of ^{13}N is fundamental in some research activities and can lead to the elucidation of processes that otherwise could not have been investigated (Hauck et al. 1994; Knowles and Blackburn 1993).

In this chapter, only the use of stable N isotopes is discussed, since their respective detection limit is low enough to monitor almost all the processes of an agricultural interest, and in particular to study fertilizer N as in the MESCOSAGR project.

5.2.1 Units of Measurement

The units of measurement for ^{15}N tracer materials are aimed at expressing the relative abundance of ^{15}N compared to ^{14}N using different notations. There are two main units of measurement; one is commonly used with ^{15}N -enriched materials,

while the other approach is more useful when natural variations in ^{15}N abundance are used to trace N.

5.2.2 ^{15}N -Enriched Tracers

The unit expresses the isotope abundance as a percentage of ^{15}N over total N (Barracough [1995](#)):

$$\text{atom}\% \ ^{15}\text{N} = \frac{^{15}\text{N}}{^{15}\text{N} + ^{14}\text{N}} \times 100.$$

It is also common to express the ^{15}N abundance as a variation with respect to N_2 natural abundance, called atom% ^{15}N excess. The $^{15}\text{N}/(^{15}\text{N} + ^{14}\text{N})$ ratio of atmospheric N_2 has been determined by Junk and Svec ([1958](#)) to be 0.003663 that is 1 ^{15}N atom every 272 ^{14}N atoms.

$$\text{atom}\% \ ^{15}\text{N excess} = \frac{^{15}\text{N}}{^{15}\text{N} + ^{14}\text{N}} \times 100 - 0.3663.$$

5.2.3 ^{15}N -Natural Abundance

The unit expresses the variation of the ^{15}N abundance with respect to a standard abundance represented by N_2 in air (Bedard-Haughn et al. [2003](#); Shearer and Kohl [1993](#)). Since the natural variations of ^{15}N abundance are low, typically 1–2% of the atmospheric N_2 (Bedard-Haughn et al. [2003](#); Shearer and Kohl [1993](#)), the unit used to express ^{15}N natural abundance is $\delta^{15}\text{N}$ (per mil ^{15}N excess, i.e., per mil variation from a standard):

$$\delta^{15}\text{N} = \frac{R(\text{sample}) - R(\text{standard})}{R(\text{standard})} \times 1,000,$$

R has been alternatively defined as:

$$R = \frac{^{15}\text{N}}{^{15}\text{N} + ^{14}\text{N}} \quad \text{or} \quad R = \frac{^{15}\text{N}}{^{14}\text{N}}.$$

The first definition is more useful in source identification studies, while the second is useful for isotope discrimination. The latter is the most commonly used (Bedard-Haughn et al. [2003](#); Mariotti et al. [1982](#)). At the level of natural

abundance, the two definitions are practically identical, as the difference in $\delta^{15}\text{N}$ using the two definitions is much smaller than the error of measurement (Barracough 1995; Hauck et al. 1994; Shearer and Kohl 1993).

5.3 Tracer Materials

To examine the N source or sink strength, flow rate, or fate in different natural or agricultural ecosystems, two alternative approaches can be used: either natural differences in ^{15}N abundance or alternatively an N source with an artificially altered ^{15}N content applied to produce a substantial difference between the tracer and the surrounding environment (Bedard-Haughn et al. 2003; Knowles and Blackburn 1993).

The natural variation of ^{15}N abundance has been used in many studies (Kerley and Jarvis 1997), although it is limited by the fact that the well-defined natural N-pools of considerably different isotopic composition need to be identified (Gerzabek et al. 2001). Bedard-Haughn et al. (2003) indicated that on average the minimum difference among sources is about 5.9%. Isotope discrimination (Sect. 5.4.1) in the soil N cycle complicates the quantification of N turnover and dynamics by using natural abundance approach (Wagner 1991).

Materials containing unnaturally high or low concentrations of ^{15}N are commonly used when natural differences are too low to be used to trace N, when the natural variability is high enough to cover any difference between sources and sink, or when processes need to be monitored over a prolonged period. In the latter case, a high gradient needs to be maintained over long periods despite a progressive re-equilibration due to N flows. While enriched materials enable the monitoring of many processes that otherwise could not be quantified or detected, natural abundance methods (NAMs) enable systems to be monitored without any disturbance. However, N cycle disturbance is usually not a problem in agro-ecosystems where N fertilization is common (Bedard-Haughn et al. 2003).

When applying artificial tracers, ^{15}N -enriched or ^{15}N -depleted tracers can be used. Depleted sources are cheaper and do not tend to contaminate laboratory equipment. Unfortunately, their tracer value is equivalent to a material containing only 0.7 atom% ^{15}N , thus N can be traced into plants only during the year of application and can be followed into the soil nitrate and organic pools only in soils with a organic N content lower than 1.5 g N kg (Hauck et al. 1994).

Two major ^{15}N -labelling techniques are routinely used to study N transformation in soil-plant systems (1) ^{15}N tracer technique and (2) ^{15}N isotope dilution technique. The first technique is based on ^{15}N labelling of a substrate pool and subsequent monitoring of the isotope's movement through the system over time. The latter technique labels a soil N pool with ^{15}N , and rates are monitored at which the N content of the pool changes and the ^{15}N atom% enrichment of the pool is diluted by ^{14}N influx (Hart and Myrold 1996).

Before beginning a new experiment, it is necessary to decide the difference in atom% needed between sources and sinks to trace N, thus the ^{15}N abundance in the labelled material. This is possible by estimating the likely degree of tracer dilution during the process monitored. For example, with many field crops, the fertilizer derived N in the plant is diluted two- to fourfold with unlabelled N derived from soil (Powlson and Barraclough 1993). Examples of calculation methods can be found in Hauck et al. (1994) and Powlson and Barraclough (1993).

5.3.1 *Samples Collection and Processing*

The need to collect representative soil and plant samples is greater when conducting field studies with labelled N than with non-labelled N, since a small amount of contamination can have drastic confounding effects on the results (Hauck et al. 1994; Powlson and Barraclough 1993; Shearer and Kohl 1993).

With the application of N isotopes in agricultural studies, plant materials and soil are commonly sampled. Plant sampling usually entails collecting the total biomass of the crop, which is then divided into its constituent plant parts. Separating plants into fractions having a relatively homogeneous N content is recommended, because different ^{15}N abundances can be found in different fractions (see isotope discrimination in Sect. 5.3.5.1). If plant parts are not mixed thoroughly, then there could be errors in the determination of atom% ^{15}N of sampled materials. The atom% ^{15}N of the entire plant can be calculated as a weighted average of the atom% ^{15}N of its individual parts on the quantity of N (kg N ha^{-1}) in the individual parts. If it is not possible to divide the plant into homogeneous tissues, then it is important to sample the heterogeneous mixture only after drying and thoroughly grinding to a particle size lower than 100 mesh (Hauck et al. 1994; Powlson and Barraclough 1993; Shearer and Kohl 1993).

During multi-season studies, special care should be taken to ensure that the sampling or harvesting operations do not cause cross-contamination among plots. Contamination can be particularly serious when large amounts of material are transported across the field (for example, maize stalks) with a relatively high ^{15}N enrichment (Hauck et al. 1994; Powlson and Barraclough 1993).

For soil sampling, a sufficient number of cores need to be mixed to accurately estimate the plot average with a given degree of precision. Examples of number of core estimations are provided by Gomez and Gomez (1984) and Hauck et al. (1994). When exploring different soil horizons, different ^{15}N enrichments are usually found. Soil near the surface usually has a much higher ^{15}N enrichment than deeper soil (Powlson and Barraclough 1993).

Sampling and material processing also need to be executed with extreme care and with suitable equipment to avoid cross-contamination. The most important rules to follow in preparing samples for analysis are (a) quantitative conversion at all steps to avoid isotopic fractionation during incomplete conversion or N loss; (b) avoidance of any contamination, especially of natural abundance samples with enriched material; (c) representative homogeneous sub-samples; (d) replicate

samples; and (e) inter-laboratory comparisons. When plant or soil samples need to be ground, disk- or ball-mills are easier to clean between subsequent samples and will usually achieve a finer grinding than hammer- or knife-mills (Powlson and Barraclough 1993).

5.3.2 Analytical Methods

Two alternative methods are available to measure ^{15}N abundance in samples: mass spectrometry and emission spectrometry. Mass spectrometry is the only sufficiently precise method for measurements at the level of natural abundance, while emission spectrometry can only be used with enriched materials. Hence, mass spectrometry is the most commonly used technique (Knowles and Blackburn 1993).

Before analysis, N in samples needs to be converted to N_2 . Two possible methods are available: the Dumas and the Rittenberg methods. The Dumas method consists in a complete combustion of the sample followed by a complete reduction of N oxides to N_2 . This method is commonly used when a mass spectrometer is coupled with an elemental analyser. The same procedure, without combustion and only with reduction, can be used for N oxides in gaseous samples (Mosier and Schimel 1993). On the other hand, the Rittenberg method consists in an oxidation of NH_4^+ to N_2 by hypobromide. When N is present in chemical forms different from ammonium, a Kjeldahl digestion with a nitrate reduction is usually carried out first (Knowles and Blackburn 1993). If the Kjeldahl method is used, corrections must be made (a) to calculate the total N in samples using the true average atomic mass of N; (b) for the extraneous N that contaminates the sample (N dissolved in sulphuric acid) and decreases the ^{15}N enrichment of a labelled sample (Powlson and Barraclough 1993). Examples of ^{15}N abundance calculations from mass/charge (m/e) ratios obtained with mass spectrometry can be found in the study of Mulvaney (1993) and from emission spectra in the study by Preston (1993).

5.3.3 Problems Linked to the Use of ^{15}N

Two phenomena usually concern isotope flows into the environment and potentially affect the goodness of rate estimates: isotope discrimination and added N interaction.

5.3.3.1 Isotope Discrimination

Isotope discrimination (also called isotope fractionation) consists in a differential reaction rate between ^{15}N and ^{14}N in biochemical or physical processes, with biotic processes generally showing a higher variability of intensity (Bedard-Haughn et al.

2003; Shearer and Kohl 1993). Usually, ^{14}N transformation is faster, leading to an ^{15}N enrichment of the reagents or original forms. Of all the consequences, the natural variability of ^{15}N abundance is a valuable example of the isotope discrimination effect. Usually, isotope discrimination is not a problem when using ^{15}N enriched materials because the variation is small compared to the difference between sources. Instead, isotope discrimination should be considered when using natural abundance, since the isotope shift is likely to be of the same order of magnitude as the difference between N sources (Barracough 1995; Hauck et al. 1976).

Isotope fractionation can be caused by both the *equilibrium isotope effect* and *kinetic isotope effect*. The *equilibrium isotope effect* occurs when the transformation rates determining the equilibrium are affected by isotopic composition. They can make a considerable contribution to variations in ^{15}N abundance in components of the N cycle. One very important example is the equilibrium between NH_3 and NH_4^+ , where ^{15}N abundance in NH_3 results approximately 20% lesser than in NH_4^+ . As a consequence, ammonia volatilization from terrestrial and aquatic ecosystems enriches residual NH_4^+ and may contribute substantially to the general elevation of ^{15}N in soil compared to atmospheric N_2 .

The *kinetic isotope effect* occurs when differences in the reaction rates of molecules bearing different isotopes result in differences in ^{15}N abundance between substrates and products. Since reactions are linked, some are not reversible, some are influenced by secondary substrates, enzymes and cofactors, and more than one reaction can compete for the same substrate, the isotope effect of a process in certain conditions may vary according to environmental conditions and be expressed only partially. The overall isotope effect of a certain process is commonly known as the “Overall Observed Isotope Effect” of the entire reaction (β_{obs}). In the soil, the combined effects of the kinetic isotopic fractionation of NH_4^+ nitrification and NO_3^- denitrification can lead to an increase in the ^{15}N abundance of soil organic N. In fact, nitrification leads to NH_4^+ enrichment (NO_3^- has a lower isotopic ratio than the NH_4^+ from which it derives); denitrification leads to a NO_3^- enrichment (more ^{14}N is lost) (Bedard-Haughn et al. 2003; Shearer and Kohl 1993). Plants usually reflect the variability of soil N, although other factors such as genotype and mycorrhizal associations also influence plant ^{15}N (Bedard-Haughn et al. 2003). Microbial processes can usually alter the isotope N composition (Macko and Estep 1984).

5.3.3.2 Added Nitrogen Interaction

The isotopic technique can also lead to an added nitrogen interaction (ANI). ANI consists in an under- or overestimation of the transformation rate of added ^{15}N into the monitored sink. It is caused by processes that lead to a substitution of the original ^{14}N by added ^{15}N in different pools from the monitored sink. Such substitution results in an ^{15}N dilution due to a greater amount of ^{14}N made available (Jenkinson et al. 1985; Powlson and Barracough 1993). Since a lower

amount of labelled N is actually available than the amount distributed, a lower transformation rate is estimated than actually occurs. ANI can be caused by processes whose rate is not determined by N availability, but by other factors. Examples are immobilization and, sometimes, denitrification. If the extent of the process is not determined by the N availability, the amount of N involved in the process is constant and will be distributed between the labelled and unlabelled N on the basis of their relative abundance. If the rate is determined by N availability, the amount of N involved in the process will be a fraction of the total available N (for example in leaching). Consequently, the amount of N subtracted will be a constant fraction of each labelled and unlabelled pool, and no variation in the relative abundance of labelled and unlabelled fertilizers will occur (Giusquiani et al. 1994; Jenkinson et al. 1985; Powlson and Barraclough 1993).

An example of ANI in the inorganic N pool of soil consists in the difference between the quantity of unlabelled inorganic nitrogen remaining in soil in fertilized and unfertilized situations. Assuming that a fixed quantity of N is immobilized, the labelled inorganic fertilizer N takes the place of the unlabelled inorganic N that would have been otherwise immobilized, thus increasing the proportion of unlabelled inorganic N remaining available in the soil for plant uptake. If the plant uptake of applied fertilizer is quantified by an ^{15}N technique, an underestimation will occur due to the greater uptake of unlabelled N. The consequence of labelled N is that its quantity retained in the total plant plus soil system will remain unaltered, while the relative amount in either plants or soil will change (Powlson and Barraclough 1993).

5.4 Nitrogen Isotope Applications: Source Identification

Nitrogen tracers have commonly been applied to accomplish three main research goals (1) to trace the N fate and flows; (2) to quantify the flow rates of different sources to a process or pool sink, also in N balance studies; (3) to study metabolic processes through the isotopic discrimination associated with them (Shearer and Kohl 1993).

In this paragraph, only the identification of the contribution of different sources to a common sink will be discussed, this estimation being the main goal of many studies evaluating the N cycle in agricultural ecosystem, as in the MESCOSAGR project. The calculation theory used to estimate the source contribution is reported along with various applications to plant–soil systems.

5.4.1 Theory of Estimate Calculation

In stable N tracer studies, only the two ^{14}N and ^{15}N isotopes are available, thus only two sources can be identified. If more than two sources are present, only the

contribution of two aggregated pools can be identified (Shearer and Kohl 1993). The fractional contribution of source A to a sink S can be calculated by a simple linear interpolation of the sink ^{15}N abundance between the two extreme poles represented by the abundance of the two sources. The following calculation is used (alternatively, atom% N or $\delta^{15}\text{N}$ can be used):

$$\% \text{N from source A} = \frac{\delta^{15}\text{N source B} - \delta^{15}\text{N sink S}}{\delta^{15}\text{N source B} - \delta^{15}\text{N source A}} \times 100.$$

In natural abundance studies, the isotopic discrimination is likely to have a strong influence on the results of the estimation, since isotopic alteration is of the same order of magnitude as the difference between sources. The closer the ^{15}N abundance of the two sources, the more important it becomes to take the isotopic discrimination into account.

Isotopic discrimination can lead not only to a shift in isotopic composition of the N actually transformed, but also to an alteration of the original ^{15}N abundance of sources as a consequence of isotope accumulation due to different transformation rates. The N source can only be identified if the ^{15}N abundance of N sources is measurably different after they have undergone isotopic discrimination. Thus, in natural abundance studies, it is fundamental that calculations refer to $\delta^{15}\text{N}$ of the sources after isotopic discrimination has altered both the N flow and the source N abundance (Shearer and Kohl 1993). When evaluating the contribution of a source to an N flow, it may also be important to evaluate the real variability of sources and sinks, not only the measurement error. Examples of calculations can be found in Shearer and Kohl (1993).

As previously explained, with only two tracers, no more than two sources can be discriminated. However, this drawback can be overcome by using the isotopes of other elements as additional tracers. Oxygen, carbon or others are commonly used (Oelmann et al. 2007). The use of more than one isotope is also useful for distinguishing between the processes that contribute to a certain product (Kool et al. 2009, 2011).

In the following sections, three applications to estimate the source contribution to a sink are presented (1) tracing N in landscapes, (2) symbiotic N_2 fixation and (3) quantification of N dynamics in soil–plant systems.

5.4.2 *Tracing N in Landscapes*

Bedard-Haughn et al. (2003) reviewed the use of ^{15}N as a tracer for N cycling in landscapes. They concluded that natural ^{15}N abundance can provide valuable estimates of the contribution of different sources or identify variation in landscape processes. Unfortunately, the large ^{15}N spatial and temporal variabilities can limit source discrimination by masking sources' differences, whereas ^{15}N -enriched

materials are widely accepted as tracers. The use of N-enriched materials is limited by their excessive cost, and, in natural ecosystems, by possible disturbances due to an uncommon introduction of N in the environment. Overall, it has been recommended the $\delta^{15}\text{N}$ approach in semi-quantitative studies of N patterns to generate new hypotheses on N cycling, while the ^{15}N -enriched method can be used to quantitatively test hypotheses (Bedard-Haughn et al. 2003).

5.4.3 N_2 Fixation

The ^{15}N tracer technique has been widely applied to estimate the leguminous N_2 fixation in natural and agricultural ecosystems (Bedard-Haughn et al. 2003; Shearer and Kohl 1993). Both the $\delta^{15}\text{N}$ - and the ^{15}N -enriched methods can be applied.

The NAM can be easily applied when the soil ^{15}N abundance available for plant uptake is sufficiently different from atmospheric N_2 . The isotope effect associated with N_2 fixation usually alters the ^{15}N abundance of atmospheric N_2 by no more than 2% (Bedard-Haughn et al. 2003; Shearer and Kohl 1993). Moreover, due to isotope discrimination, the ^{15}N abundance of plant-available soil N is different from soil total N. For a correct quantification, a non- N_2 -fixing reference plant can be grown on the same soil and its ^{15}N abundance analysed. Similarly, isotopic discrimination during N_2 fixation can be assessed by growing a legume hydroponically (Shearer and Kohl 1993).

The ^{15}N -enriched method can be applied following two different procedures depending on the objective of the research experiment (1) growing the legume in an $^{15}\text{N}_2$ -enriched atmosphere or (2) on an ^{15}N -enriched soil after fertilization. If an $^{15}\text{N}_2$ -enriched atmosphere is selected, plants fix N_2 with a greater ^{15}N content than that soil. The extent to which soil N is diluted by this ^{15}N enrichment in a fixing plant reflects the magnitude of fixation. The $^{15}\text{N}_2$ -enriched atmosphere method has limitations in terms of being (a) technically difficult (to prevent leaks and maintain normal environmental conditions); (b) a short-term kinetic measurement that is not useful for long-term integrated quantification of N_2 fixation (Warembourg 1993).

The ^{15}N -enriched fertilizer method, also known as ^{15}N isotope dilution method, consists in adding ^{15}N to soil. Unlike the $^{15}\text{N}_2$ -enriched atmosphere method, plants absorb N from soil containing more ^{15}N content than atmosphere. The ^{15}N dilution of atmospheric N reflects the magnitude of fixation. The main limitation is the need to estimate the ^{15}N abundance of the N uptake by plants from soil through the analysis of ^{15}N abundance in a reference plant. This kind of quantification is necessary since the addition of a labelled fertilizer makes difficult to assume a uniform mixing with soil N.

Various calculations are available when natural abundance or ^{15}N isotope dilution methods are applied. When a reference plant is used, the following model is applied (Shearer and Kohl 1993; Hauggaard-Nielsen et al. 2003, 2009):

$$\%N \text{ from } N_2 = \frac{\delta^{15}N \text{ reference plant} - \delta^{15}N \text{ fixing plant}}{\delta^{15}N \text{ reference plant} - \delta^{15}N \text{ fixing plant grown hydroponically}} \times 100.$$

A simplified calculation for the isotope dilution method accounts for the isotope discrimination (Warembourg 1993):

$$\% N \text{ from } N_2 = \frac{\text{atom}\%^{15}N \text{ fixing plant}}{\text{atom}\%^{15}N \text{ reference plant}} \times 100.$$

5.4.4 Nitrogen Dynamics in Soil–Plant Systems

The ^{15}N tracer technique can be applied to evaluate both the net/gross mineralization rates of indigenous or added N, and the efficiency of plants to assimilate added N (Barraclough 1995). If the objective is to estimate the plant assimilation of added N, it is usually not convenient to use N tracers, and a lower cost alternative is to apply non-isotopic techniques (Powlson and Barraclough 1993). There are two methods:

- (a) *Control plot method*: N uptake of the unfertilized crop is subtracted from that of the N fertilized crop. This method has the disadvantage that the N uptake of a crop that has not received N is fundamental in the calculation. A bias could thus occur as it is assumed that the N uptake from soil is the same in both unfertilized and fertilized crops (Powlson and Barraclough 1993; Wivstad 1999).
- (b) *Regression method*: the uptake efficiency of fertilizer N is calculated as the slope of regression curve for crop N uptake when several different fertilizer N rates are applied (Wivstad 1999).

In the following sections, estimations of the gross mineralization and nitrification rates as well as the net mineralization and plant assimilations are reviewed.

5.4.4.1 Gross Mineralization and Nitrification Rates

Rates of gross N mineralization and nitrification can be estimated by using an ^{15}N tracer based on two different approaches. The first (analytical method) refers to the isotope dilution technique and analytical equations that relate changes in ^{15}N abundance of a labelled ammonium or nitrate pool to the rate of mineralization or nitrification for short-term estimates. The second (numerical method) refers to simulation models of the soil–plant nitrogen cycle and uses ^{15}N data (such as ^{15}N

abundance in soil mineral N), being preferable for long-term evaluations (Rütting and Müller 2007). The pool dilution method (isotope dilution) is based on a first-order decay equation modelling the decrease of ^{15}N concentration in NH_4^+ (gross mineralization) or NO_3^- (gross nitrification) pool, due to concurrent dilution with newly produced NH_4^+ or NO_3^- and input in other pools (Perelo et al. 2006). This method has been applied to different studies, including crop residue mineralization and their interaction with soil organic N turnovers (Watkins and Barraclough 1996).

5.4.4.2 Net Mineralization and Plant Assimilation

Net rates of mineralization and plant assimilation can be estimated by monitoring ^{15}N abundance in sources and in the final sink. Despite the unique possibility of tracing N in all the studied systems, some problems arise with the nitrogen added to soil and the need to determine ^{15}N abundance of plant available N. This latter problem can be solved by growing the same plant in a non-fertilized control experiments, as illustrated for the case of reference plants in N_2 -fixation studies. The former problem has often been considered null (Giusquiani et al. 1994).

Different equations have been developed as functions of research assumptions. The same approach can be used to calculate the fractional contribution of an N source (reported here as *labelled fertilizer*) to total N content in either a plant (reported here as *crop*) or a soil (in substitution of the plant N content).

The following are the most commonly used equations presented in the order of increasing simplicity of application and increasing assumptions required:

1. Equation (5.1) takes into account both soil ^{15}N abundance and isotopic fractionation during plant uptake of soil and fertilizer N. It can be used both in the studies dealing with natural abundance ($\delta^{15}\text{N}$) and ^{15}N -enriched fertilizer (atom% ^{15}N):

%N from fertilizer

$$= \frac{\delta^{15}\text{N non-labelled crop} - \delta^{15}\text{N-labelled crop}}{\delta^{15}\text{N non-labelled crop} - \delta^{15}\text{N crop grown only on labelled fertilizer}} \times 100. \quad (5.1)$$

It is the most accurate approach as it takes into account all possible isotope discrimination effects during N uptake. However, its great limitation is the requirement to grow plants only with N from fertilizer.

2. Equation (5.2) takes into account both the isotopic fractionation and soil ^{15}N abundance. It assumes that no isotopic fractionation occurs during fertilizer N uptake or that it does not discriminate between the non-labelled plant and the fertilizer. With respect to (5.1), it has the great advantage that it is not necessary

to grow plants only with N from fertilizer. It can be used both in the studies dealing with natural abundance ($\delta^{15}\text{N}$) and ^{15}N -enriched fertilizer (atom% ^{15}N) (Hauck and Bremner 1976; Shearer and Kohl 1993):

$$\% \text{N from fertilizer} = \frac{\delta^{15}\text{N non-labelled crop} - \delta^{15}\text{N-labelled crop}}{\delta^{15}\text{N non-labelled crop} - \delta^{15}\text{N-labelled fertilizer}} \times 100. \quad (5.2)$$

When referring to soil, (5.1) and (5.2) are equivalent.

3. Equation (5.3) takes into account both isotopic fractionation and soil ^{15}N abundance. It is similar to (5.2), but the soil ^{15}N is considered to be like atmospheric N_2 (Hauck and Bremner 1976; Powlson and Barraclough 1993)

$$\% \text{N from fertilizer} = \frac{\delta^{15}\text{N-labelled crop} - \delta^{15}\text{N control crop}}{\delta^{15}\text{N-labelled fertilizer}} \times 100. \quad (5.3)$$

Equation (5.3) is used only in the studies dealing with ^{15}N -enriched fertilizer, while for all other applications (5.2) is recommended.

4. Equation (5.4) does not take into account the isotopic fractionation during soil N uptake, but considers soil ^{15}N abundance. $\delta^{15}\text{N}$ soil is determined before fertilizer addition (Bedard-Haughn et al. 2003; Hauck et al. 1994):

$$\% \text{N from fertilizer} = \frac{\delta^{15}\text{N-labelled crop} - \delta^{15}\text{N soil}}{\delta^{15}\text{N-labelled fertilizer} - \delta^{15}\text{N soil}} \times 100. \quad (5.4)$$

It can be used both in the studies dealing with natural abundance ($\delta^{15}\text{N}$) and ^{15}N -enriched (atom% ^{15}N) fertilizer. When referring to soil, (5.1), (5.2) and (5.4) are equivalent.

5. Equation (5.5) does not take into account either isotopic fractionation or a possible soil isotopic enrichment/depletion of soil N (Powlson and Barraclough 1993; Wivstad 1999). It is the simplest equation. It can be applied only in the studies dealing with N-enriched fertilizer, in which the fertilizer ^{15}N abundance is very different from atmospheric abundance, in order to cover any isotope discrimination effect or soil N variability. In fact, isotopic fractionation can have a very small influence with 1–10 atom% labelled materials (Barraclough 1995).

$$\% \text{N from fertilizer} = \frac{\text{atom\% } ^{15}\text{N excess labelled crop}}{\text{atom\% } ^{15}\text{N excess labelled fertilizer}} \times 100. \quad (5.5)$$

Section 5.6 provides an exemplification (part of the MESCOSAGR project) of the use of an ^{15}N tracer technique to quantify the relative contribution to plant uptake of SOM and mineralization of added organic fertilizer.

5.5 Carbon Isotope Approach to Study Root Deposition

Three stable isotopic techniques are commonly used to estimate C inputs in soil by plants (1) the pulse labelling method, (2) the continuous labelling method (CLM), and (3) the ^{13}C NAM.

5.5.1 Pulse Labelling Method

The pulse labelling method (PLM) is based on the artificial labelling of plants. The technique can be roughly divided into three methods based on the levels of ^{13}C enrichment achieved in the plant–soil system: natural abundance (Heim and Schmidt 2007; Klumpp et al. 2007; Thornton et al. 2004), near natural abundance (50–500%) (Evershed et al. 2006; Leake et al. 2006) or (highly) enriched $>500\%$ (Zak and Kling 2006). Shoots are exposed to CO_2 in an atmosphere labelled with ^{14}C , ^{13}C or ^{11}C . The shoots assimilate the labelled C and translocate a part of it into the soil. This C is incorporated into the root tissue, exuded as high and low-molecular-weight organic substances, sloughed as cell tissues by root elongation and released as CO_2 derived from root respiration. Hence, the entire labelled C later found in all soil pools or evolved as CO_2 from the soil is plant-derived. This enables the C input by plants in soil to be calculated on the background of soil organic C, which remains unlabelled. The calculations are similar to those used for natural isotope labelling outlined below (the third method). The fractional input (F^*) of C from the new ^{13}C natural source in the existing soil C pool (or constituents) can be estimated using a linear mixing model as follows:

$$F^* = \frac{\delta \text{ final} - \delta \text{ initial}}{\delta \text{ source} - \delta \text{ initial}},$$

where F^* is the proportion of new C present in soil, $\delta \text{ source}$ is the $\delta^{13}\text{C}$ or atom% ^{13}C of the source C applied to soil, and $\delta \text{ final}$ and $\delta \text{ initial}$ are the initial and final $\delta^{13}\text{C}$ or atom% ^{13}C of soil C pool at the beginning and end of the experimental period.

Near natural or high enrichment labelled approaches generally use artificially labelled plant materials (Leake et al. 2006) or commercially available ^{13}C -labelled substrates (Evershed et al. 2006; Zak and Kling 2006) to trace C flows. Due to high costs to produce labelled plant material or buy expensive ^{13}C -labelled compounds, most of these approaches are laboratory based (e.g., Evershed et al. 2006) or rely on small field experiments (e.g., Leake et al. 2006; Zak and Kling 2006). Experiments using artificial labelling methods are generally conducted for short periods of time (i.e., weeks to months), hence they generally only completely label those soil components which have a relatively high turnover rate (e.g., soil microbial community and water soluble carbon).

In the case of pulse labelling, the shoots assimilate the labelled CO_2 for only a short period, even only once during the whole plant growth. In continuous labelling technique, the plants assimilate labelled CO_2 over a long period. Many different experimental systems for pulse and continuous labelling of plants have been described (e.g., Sauerbeck and Johnen 1976; Whipps and Lynch 1983; Shepherd and Davies 1993; Cheng et al. 1993; Jenkinson et al. 1999; Swinnen et al. 1994). The results obtained by pulse labelling correspond to the relative distribution of assimilated C at the moment of labelling and do not reflect the distribution of total unlabelled C in different plant parts. The total amount of C assimilated by the plant is unknown and can only be roughly calculated.

The most important limitation of pulse labelling is that the results of C allocation observed at a specific time or growth stage cannot be directly transferred to the whole growth period. However, a series of labelling pulses applied at regular intervals during plant growth have been found to provide a reasonable estimate of cumulative belowground C input (Jenkinson et al. 1999; Swinnen et al. 1994; Kuzyakov and Schneckenberger 2004).

5.5.2 Continuous Labelling Method

In the case of continuous labelling, the total amount of assimilated C is known. In addition, the distribution of labelled C corresponds to the distribution of total C, as long as the labelling is applied from first leaf emergence to harvest time (the specific ^{14}C activity or ^{13}C abundance is equal in all plant parts). Therefore, CLM is particularly appropriate for estimating the amount of total C transferred by plants into soil and belowground pools during the all labelling period (Meharg 1994), and is also useful to distinguish root-derived from SOM-derived CO_2 (Johnen and Sauerbeck 1977; Whipps 1990). Continuous labelling requires special equipment to expose plants over a long period to $^{14}\text{CO}_2$ with constant ^{14}C specific activity, or to $^{13}\text{CO}_2$ with ^{13}C constant enrichment. In addition, the air temperature and moisture conditions must be controlled inside the labelling chamber.

For both pulse and CLMs, radioactive ^{14}C has been used in most studies. This preferential use of ^{14}C is based on higher sensitivity, lower purchasing and analysis costs, and easier sample preparation than for ^{13}C or ^{11}C . Since ^{11}C has a short half-life (20.4 min), only ^{14}C and ^{13}C are appropriate for continuous labelling.

Unlike in traditional methods, in tracer techniques, the amount of tracer that enters the system is known exactly and it is possible to exactly calculate the balance of C in the atmosphere–plant–soil system, as well as the losses from the system. Traditional methods are less accurate and can only be used to calculate the distribution of C among different C pools.

5.5.3 Natural Abundance Method

The use of ^{13}C NAM in soil studies provides a highly accurate method to trace carbon transfer among pools, and has been successfully used in studies on SOC dynamics (Martel and Paul 1974; Nissenbaum and Schallinger 1974; Leavitt et al. 1994; Paul et al. 2001; Stevenson et al. 2005). NAM is based on the discrimination of ^{13}C and ^{12}C isotopes during CO_2 assimilation by plants due to different photosynthesis pathways, which lead to plants with distinct $\delta^{13}\text{C}$ values. Plants with a C_3 photosynthetic pathway have $\delta^{13}\text{C}$ values ranging from about -32 to -22% (mean -27%), whereas those with a C_4 pathway range from -17 to -9% (mean -13%) (Boutton et al. 1998). The isotopic composition of soil organic C reflects the plant materials from which it is derived. The NAM is based on the cultivation of C_3 plants in a soil developed under C_4 vegetation, or vice versa, and the estimation of rhizodeposition according to the $\delta^{13}\text{C}$ value in soil C pools or CO_2 evolved from soil. This method can be considered as a variation of continuous labelling, because plants and soils are permanently labelled. However, the labelling of plants and soils is the result of natural processes, rather than the artificial procedures used in the case of pulse or CLMs described above. NAM can easily be used under field conditions (Rochette and Flanagan 1998) because special equipment for plant labelling and separation from atmosphere is not necessary. The latter feature and future development of mass-spectrometry will promote the use of this method in forthcoming investigations.

The natural ($\delta^{13}\text{C}$) isotopic difference (about 14%) between C_3 and C_4 plants enables new carbon derived from one pathway (e.g., C_3) to be traced in SOM that is derived from plants having the other pathway (Balesdent and Mariotti 1996; Gleixner et al. 2002; Lobe et al. 2005).

Balesdent and Mariotti (1996) proposed a method to calculate the replacement of old soil carbon by the new vegetation carbon. They propose to derive the contribution of plant B to the total C content using the expression:

$$F = C_B/C = \frac{(\delta_{AB} - \delta_A)}{(\delta_B - \delta_A)},$$

where F is the fraction of new carbon in soil, and A and B represent the different photosynthetic pathway types (e.g., C_3 and C_4). $C = C_A + C_B$ is the total soil carbon content; C_A and C_B are equal to the organic carbon contents from the old (A) and new (B) vegetation, and δ_{AB} is the isotopic composition of the soil C under mixed vegetation:

$$\delta_{AB}(C_A + C_B) = \delta_{AB}(C) = \delta_A C_A + \delta_B C_B,$$

δ_B and δ_A are the $\delta^{13}\text{C}$ values of vegetation A and B.

Because δ_A and δ_B cannot be measured directly in the mixed cropping system, δ_B is estimated by the $\delta^{13}\text{C}$ value of the new vegetation ($\delta_{\text{VEG B}}$), replacing also

the δ_A values with $\delta^{13}\text{C}$ of a control site that still has the original vegetation ($\delta_{\text{VEG A}}$) and soil $\delta^{13}\text{C}_A$ value by that of the control soil ($\delta_{\text{REF A}}$), respectively (Balesdent and Mariotti 1996). Finally the new portions of vegetation B are estimated from:

$$F = \frac{(\delta_{\text{AB}} - \delta_{\text{REF A}})}{(\delta_{\text{VEG B}} - \delta_{\text{VEG A}})}.$$

The limitations of the ^{13}C NAM are caused by soil–plant pairs. Situations, where C_3 plants grow in a C_4 soil, or vice versa, are unnatural. Hence, this method is restricted to places where soils developed under C_3 vegetation allow the growth of C_4 plants and vice versa. Moreover, high-resolution and high-sensitivity mass-spectrometry is necessary for ^{13}C analyses because a maximal range of only 14% is available for all variations of the $^{13}\text{C}/^{12}\text{C}$ ratio. At the same time, the variability of $\delta^{13}\text{C}$ values in soil or plant is about $\pm 1\text{--}2\%$ (Cheng et al. 1993). For the latter two reasons, only a rough estimation of rhizodeposition in soil and in carbon pools with large exchange rates with root-derived C is possible (e.g., microbial biomass, dissolved organic C, active pools of SOM, etc.). Finally, a limitation of all methods based on C tracers is that organic C-pools may interact with inorganic C-pools in soil (carbonates and bicarbonates).

5.6 Nitrogen Tracers in the MESCOSAGR Project

A study on the stable isotope ^{15}N was set up to (1) evaluate the contribution of compost to N nutrition of maize (Ndfc) as well as the fertilizer use efficiency (%FUE) of compost-derived nitrogen for 3, 2 and 1 years amendments; (2) assess the ability of an ^{15}N enrichment technique to trace the N flows from compost to maize and separate the effects of different amendment years even after repeated application; (3) quantify the incorporation of N from compost into SOM.

^{15}N -labelled compost (Table 5.1) was prepared at the University of Basilicata and applied to the unlabelled soil at the experimental field of the University of Torino each year (TO site already described in Chap. 3) where maize (*Zea mays* L.) was grown in a 3-year experiment (2006, 2007, 2008). The experimental design consisted of four treatments (Table 5.2), laid out with four replicates in a complete

Table 5.1 Chemical properties of composts used each year

Year	Total N (kg ha^{-1})	C/N	$\delta^{15}\text{N}$ (%)	Lignin N (%)	Lignin $\pm$ cellulose N (%)	C/P
I – 2006	130	12.4	242.0	6.8	11.8	39.8
II – 2007	166	37.7	193.1	13.7	41.8	49.6
III – 2008	128	24.3	136.6	11.3	23.4	59.6

Table 5.2 Description of plot treatments (A–D) and nitrogen (kg ha^{-1}) added in each plot treatment in different forms (labelled compost or unlabelled urea)

Year	Experimental plot			
	A	B	C	D
I – 2006	^{15}N compost (COM1) (148)	Urea (130)	Urea (130)	0
II – 2007	^{15}N compost (COM2) (166)	^{15}N compost (COM2) (166)	Urea (130)	0
III – 2008	^{15}N compost (COM3) (128)	^{15}N compost (COM3) (128)	^{15}N compost (COM3) (128)	0

block design. The treatments were applied to individual plots of 12 m^2 , selected within a main plot of 48 m^2 .

Maize epigeal biomass production (grains, stalk plus leaves and cob plus husks) was determined each year, while root biomass production was determined only 1 year and the shoot/root ratio in the other 2 years. Each year aboveground and root biomass samples were analysed for total N content (Carlo Erba NC2500 elemental analyser) and atom% ^{15}N excess (Finnigan Delta-Plus isotope ratio mass spectrometer).

5.6.1 Contribution to Maize Nitrogen Nutrition of Compost

The fraction N taken up by maize and deriving from compost ($\%N_{\text{pfc}}$) was estimated following a modified model [(5.6), consisting of an application of (5.1)] derived from Shearer and Kohl (1993) and taking into account the isotopic discrimination during plant absorption of soil and fertilizer N:

$$\%N_{\text{pfc}} = \frac{\text{atom\% } ^{15}\text{N excess labelled maize} - \text{atom\% } ^{15}\text{N excess non-fertilized maize}}{\text{atom\% } ^{15}\text{N excess maize grown on labelled fertilizer} - \text{atom\% } ^{15}\text{N excess non-fertilized maize}} \times 100. \quad (5.6)$$

Isotopic discrimination during compost mineralization and plant N uptake was evaluated for one vegetative cycle of maize fertilized only with compost in a pot experiment. Since the compost applied differed over the 3 years, the calculated shift of isotope percentage was used in the other 2 years to estimate the atom% ^{15}N excess of maize grown only on labelled compost. The ANI was considered null (Powlson and Barraclough 1993) and the contribution of seed nitrogen was considered insignificant.

As compost was repeatedly added, the residual compost mineralization in subsequent years of application was estimated by comparing the $\%N_{\text{pfc}}$ of

treatments and assuming that the addition of new compost did not influence the mineralization rate of residual compost.

5.6.2 Soil Sampling, Aggregate Fractionation and Isotope Determination

Soil samples were collected from topsoil (0–30 cm) and the method described by Kemper and Rosenau (1986) and Spaccini et al. (2004) was used to separate water-stable aggregates. Twenty grams of <4.75 mm air-dried soil sample was put in the topmost sieve of a nest of three sieves of 1.00, 0.50 and 0.25 mm mesh size and pre-soaked in distilled water for 30 min. Thereafter the nest of sieves and their content were oscillated vertically in water 20 times using a 4-cm amplitude at the rate of one oscillation per second. Care was taken to ensure that soil particles on the topmost sieve were always below the water surface during each oscillation. After wet-sieving, the soil materials left on each sieve and the unstable (<0.25 mm) aggregates were quantitatively transferred into beakers, dried in the oven at 50°C for 48 h, weighed and stored for the analyses of total and organic C, and total N. The percentage ratio of the aggregates in each sieve represented the water-stable aggregates in the following size classes: 4.75–1.00, 1.00–0.50, 0.50–0.25 and <0.25 mm. The mean-weight diameter (MWD) of water-stable aggregates was calculated as μm

$$MWD = \sum_{i=1}^n X_i \times W_i,$$

where X_i is the mean diameter of the i th sieve size, and W_i the weight of total aggregates in the i th fraction.

After aggregate fractionation, soil aggregates were finely ground in an agathe mortar to a fine powder (<200 mesh), and duplicate subsamples (~25 mg) of soil were analysed for $\delta^{15}\text{N}$ using a Finnigan Delta-Plus isotope ratio mass spectrometer linked to a Carlo Erba NC2500 elemental analyser located at the University of Basilicata.

The proportion (f) of soil N derived from the ^{15}N -labelled compost was calculated using the ^{15}N atom% values of the ^{15}N -enriched samples against the ^{15}N natural abundance samples (control-D) by the isotope dilution method:

$$f = \frac{\text{atom}\% \text{ } ^{15}\text{N} \text{ excess sample} - \text{atom}\% \text{ } ^{15}\text{N} \text{ excess natural abundance}}{\text{atom}\% \text{ } ^{15}\text{N} \text{ excess labelled material} - \text{atom}\% \text{ } ^{15}\text{N} \text{ excess natural abundance}},$$

where ^{15}N sample = ^{15}N atom% for the sample of interest; ^{15}N -labelled material = ^{15}N atom% of compost; ^{15}N natural abundance = ^{15}N atom% of soil from the same plot collected before the addition of ^{15}N -labelled compost.

5.6.3 Results and Discussion

5.6.3.1 Enrichment by ^{15}N in Maize Plant

The effect of the ^{15}N -labelled compost amendments for ^{15}N enrichment in plants was different among treatments after 3 years of experiments (Fig. 5.1). Results indicate that the $\delta^{15}\text{N}$ enrichment in maize plants was larger for all compost treatments than for controls. Moreover, the $\delta^{15}\text{N}$ of maize increased by continuous compost application each year. The $\delta^{15}\text{N}$ values in grains of treatment A was significantly greater than those of treatments B, C and D and there were significant differences among all treatments.

The first year mineralization of compost was quantified to be about 20% of applied N, with decreasing values in the second and third subsequent years. A great variability was found in the compost mineralization rates in the first year depending on compost maturity and composition (data not shown). These results are in line with the findings of Sikora and Enkiri (2001), who observed a 25% total availability of added compost. Similarly, Hargreaves et al. (2008) indicated a 10–22% availability of compost N.

5.6.3.2 $\delta^{15}\text{N}$ in Different Soil Aggregate Fractions

Table 5.3 shows the values of soil $\delta^{15}\text{N}$ (%) in different treatments and soil aggregates after a continuous 3-year experiment.

Abundance of stable N isotopes varied among soil aggregate fractions and treatments after three experimentation years. The ^{15}N values were more variable

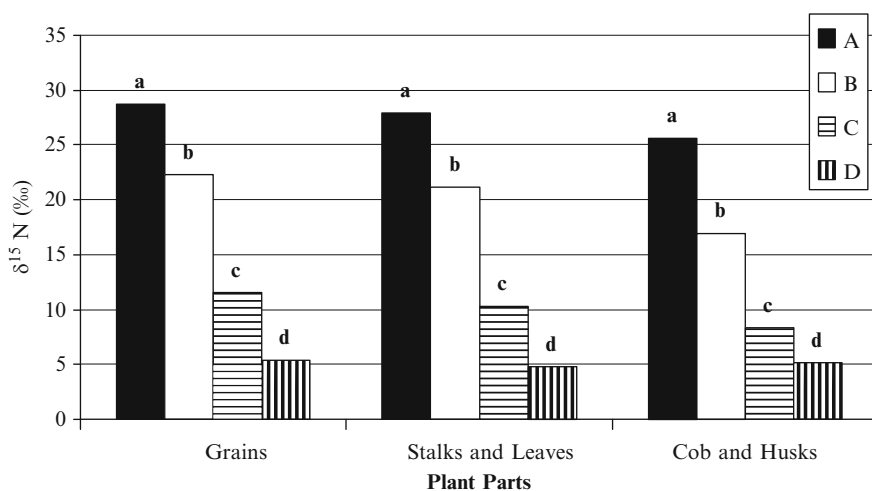


Fig. 5.1 Changes in $\delta^{15}\text{N}$ (‰) values of aboveground maize parts as affected by labelled organic amendment in different treatment plots (a–d). The bars with different letters within each plant part are statistically significant at $P < 0.05$

Table 5.3 Mean $\delta^{15}\text{N}$ (‰) values in bulk topsoil (0–30 cm) and aggregate sizes (mm) after the third experimentation year

Soil sample	Experimental plots							
	A (III)		B (III)		C (III)		D (III)	
	$\delta^{15}\text{N}$	$\pm\sigma$	$\delta^{15}\text{N}$	$\pm\sigma$	$\delta^{15}\text{N}$	$\pm\sigma$	$\delta^{15}\text{N}$	$\pm\sigma$
BULK	12.35	1.78	9.13	1.25	5.14	1.10	4.31	1.38
4.75–1	21.38	12.90	14.31	3.47	6.17	1.67	2.95	0.38
1–0.5	11.04	2.60	8.93	4.78	4.78	0.29	2.87	1.76
0.5–0.25	9.94	1.62	5.67	0.45	5.92	1.20	3.34	0.72
<0.25	8.39	0.63	5.33	2.24	4.44	1.95	2.43	1.69

Table 5.4 Mean $\delta^{15}\text{N}$ (‰) values in treated plot A for bulk topsoil (0–30 cm) and aggregate sizes (mm) over experimentation years (0, I, II and III years)

Soil sample	A (0)		A (I)		A (II)		A (III)	
	$\delta^{15}\text{N}$	$\pm\sigma$	$\delta^{15}\text{N}$	$\pm\sigma$	$\delta^{15}\text{N}$	$\pm\sigma$	$\delta^{15}\text{N}$	$\pm\sigma$
BULK	4.31	1.38	10.66	2.20	7.65	1.35	12.35	1.78
4.75–1	2.95	0.38	9.54	4.85	7.74	1.89	21.38	12.90
1–0.5	2.87	1.76	6.61	2.12	6.94	3.07	11.04	2.60
0.5–0.25	3.34	0.72	7.20	2.42	8.67	0.54	9.94	1.62
<0.25	2.43	1.69	4.57	0.70	8.00	1.46	8.39	0.63

Table 5.5 Soil $\delta^{15}\text{N}$ (‰) enrichment in bulk topsoil (0–30 cm) and aggregate sizes (mm) as affected by ^{15}N -labelled compost additions in different treatment plots

Soil sample	Experimental plots							
	(A) 2006		(B) 2007		(C) 2008		(0) Control	
	$\delta^{15}\text{N}$	$\pm\sigma$	$\delta^{15}\text{N}$	$\pm\sigma$	$\delta^{15}\text{N}$	$\pm\sigma$	$\delta^{15}\text{N}$	$\pm\sigma$
BULK	10.66	2.20	5.30	1.05	5.14	1.10	4.31	1.38
4.75–1	9.54	4.85	3.23	1.32	6.17	1.67	2.95	0.38
1–0.5	6.61	2.12	5.21	1.33	4.78	0.29	2.87	1.76
0.5–0.25	7.20	2.42	5.15	1.32	5.92	1.20	3.34	0.72
<0.25	4.57	0.70	4.38	0.58	4.44	1.95	2.43	1.69

in A and B treatments than in C and D treatments. In most cases the $\delta^{15}\text{N}$ values increased with increasing soil aggregate size (Table 5.4).

Data indicate that ^{15}N enrichment in bulk soil and all soil aggregates increased with time, as compared to plot soil at time 0. The soil ^{15}N enrichment following yearly ^{15}N -labelled compost amendment in different plots (A–C) over time is reported in Table 5.5. Different enrichments were obtained, possibly due to different chemical compositions of added composts (Table 5.1).

The macro-aggregate fraction was shown to be very sensitive and responsive to management (Elliott 1986; Six et al. 2000). This phenomenon was observed also in our study, where most changes for the ^{15}N -labelled material were associated to macro-aggregates. Smaller and slower changes in soil ^{15}N organic matter were observed with micro-aggregates and silt-and-clay fractions.

Table 5.6 Percent of soil-N derived from ^{15}N -labelled compost materials in bulk topsoil (0–30 cm) and aggregate-sizes (mm) in different treatment plots over the experimentation time

Soil samples	Experimental plots		
	(A) COM1, 2006	(B) COM2, 2007	(C) COM3, 2008
2006			
BULK	2.68		
4.75–1	2.76		
1–0.5	1.57		
0.5–0.25	1.62		
<0.25	0.89		
2007			
BULK	0.99	0.52	
4.75–1	1.89	0.15	
1–0.5	0.73	1.22	
0.5–0.25	1.49	0.94	
<0.25	1.52	1.01	
2008			
BULK	1.38	2.12	0.61
4.75–1	3.11	4.35	2.36
1–0.5	0.91	2.21	1.41
0.5–0.25	1.81	0.14	1.90
<0.25	1.29	0.47	1.48

Nitrogen added with compost is bound to be progressively incorporated in the SOM. The use of an ^{15}N tracer enabled to monitor the SOM evolution under compost addition despite the short duration of the experiment (only 3 years). The percent of soil nitrogen derived from ^{15}N -labelled compost in bulk soil and aggregate-sizes is reported in Table 5.6. Estimation of total compost-derived N sequestered in soil resulted to be 34.2, 38.2 and 42.5 percent of total N added with compost, for 1-year amendment (148 kg N ha^{-1}), 2-year amendments (314 kg N ha^{-1}), and 3-year amendments (442 kg N ha^{-1}), respectively (data not shown).

5.7 Carbon Tracers in the MESCOSAGR Project

5.7.1 Site Description and Experimental Design

Field experiments were conducted during the 2006, 2007 and 2008 seasons growing at the Agronomic Institute, for industrial crops (CRA-Ort) of Battipaglia (Salerno, Italy). The set-up consisted of a randomized complete block design with four replications, and four treatments (Table 5.7) with a total of 16 plots of $5 \times 8 \text{ m}$.

The adopted crop was sorghum [*Sorghum bicolor* (L.) $\times$ *S. sudanense* (Piper) Stapf.; cv: BMR333], planted by hand in rows with a density of 20 plant/m^2 . The plots were weeded by hand twice a year before incorporating compost (0–15 cm) or

Table 5.7 Description of soil treatments

Treatments	Fertilization	Dose N (kg ha ⁻¹)	Irrigation	Sorghum
TRA	Urea	130	Yes	Yes
CPT1	Compost	130	Yes	Yes
CPT2	Compost	260	Yes	Yes
0-N	NO	0	Yes	Yes

adding nitrogen fertilizer (TRA plot), and sowing. Sorghum was harvested each year in September.

5.7.2 Soil Sampling and Sample Treatment

In order to study the plot spatial variation of soil C stable isotope ($\delta^{13}\text{C}$), 36 soil samples (0–15, 15–30 and 30–60 cm depth) were collected, before starting the field trial, from 4 transects spaced 10 m apart with 3 sampling points along each transect (7 m between sampling points).

For each treatment, soil samples were also collected from 0–15, 15–30, 30–60, 60–90, 90–120, 120–150, 150–180 and 180–210 cm depths either before planting or after harvesting in the 2007, 2008 and 2009 growing seasons. All samples were air-dried and ground to a fine power. To avoid the possible misleading influence of inorganic carbon during determination of the isotopic signature of organic C, all carbonates were removed prior to the isotopic analysis (Harris et al. 2001). Subsamples of (1.5 mg) soil were placed in open Ag-foil capsules (4 by 6 mm). Silver capsules were required because Sn capsules disintegrate when exposed to HCl vapour. The capsules were placed in wells of a microtitre plate, sufficient water was added to each capsules (4 μL) to moisten the soil near to field capacity. The microtitre plate was then placed inside a vacuum desiccator (5 L) with a beaker (150 ml) filled with 100 ml of 12-M HCl. Samples were exposed to HCl vapour for 6 h and were then oven-dried at 60°C and analysed for $\delta^{13}\text{C}$ signature. Duplicate soil subsamples (1.5 mg) were analysed for $\delta^{13}\text{C}$ using a Finnigan Delta-Plus isotope ratio mass spectrometer coupled to a Carlo Erba NC2500 elemental analyser. A Carlo Erba NC2500 elemental analyser was also used to analyse total and organic carbon of soil.

5.7.3 Plant Tissue Treatment and Analysis

Plant samples (leaves, stems and roots) were oven-dried at 70°C, ground to a fine powder with a ball mill and weighed in tin capsules. The samples (3.2-mg leaves, 6-mg stems and 8-mg roots) were then burnt in an elemental analyser (Carlo Erba Instruments NCS 2500) equipped with an auto-sampler carousel and controlled by

EAGER 2000 software. The $\delta^{13}\text{C}$ values were measured using a Finnigan Delta-Plus isotope ratio mass spectrometer coupled with autoanalyser and using CO_2 as a reference gas.

The isotope analyses were expressed as $\delta^{13}\text{C}$ values

$$\delta^{13}\text{C} = \frac{R_{\text{sample}}}{R_{\text{standard}} - 1} \times 1,000,$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ is for either the sample or the reference standard. The $\delta^{13}\text{C}$ values were referred to PeeDee Belemnite (PBD) as standard. The standard deviation of duplicate samples for $\delta^{13}\text{C}$ was ± 0.3 .

The fraction of soil C derived from the new sorghum residue input (f_{new}) was calculated with the isotopic mixing model (Leavitt et al. 1994) as

$$f_{\text{new}} = \frac{\delta^{13}\text{C}_{\text{sample}} - \delta^{13}\text{C}_{\text{old}}}{\delta^{13}\text{C}_{\text{new}} - \delta^{13}\text{C}_{\text{old}}},$$

where $\delta^{13}\text{C}_{\text{sample}}$ and $\delta^{13}\text{C}_{\text{old}}$ are the isotopic signatures of SOC after and before the sorghum experiment, respectively. $\delta^{13}\text{C}_{\text{new}}$ represents the $\delta^{13}\text{C}$ value of new input C from C_4 -sorghum residues. In this calculation, we assumed that no isotopic discrimination occurred during the microbial decomposition of SOC and sorghum residues.

5.7.4 Results and Discussion

5.7.4.1 Carbon Isotopic Signature in the Sorghum Plants

Figure 5.2 shows the average isotopic signature of various parts of the sorghum plant, as evaluated at the end of the vegetative cycle. Generally, data indicated that the roots were more ^{13}C -enriched than the grain and leaves, and in the first 15 cm of roots appeared more ^{13}C -enriched than at deeper soil depths. Our results are consistent with previous studies on isotope ratios in plant biomass which showed that roots are generally more ^{13}C -enriched than leaves (Schweizer et al. 1999; Brugnoli and Farquhar 2000). It is to be noted that our $\delta^{13}\text{C}$ value for the stem was not statistically different from that of roots (Fig. 5.2).

5.7.4.2 Initial Variability of Soil $\delta^{13}\text{C}$

The average value of total $\delta^{13}\text{C}$ value in the field site before the experiment was -23.003% , with a low variability within the soil profile (Table 5.8). We observed a low $\delta^{13}\text{C}$ variability in the 0–60 cm layer with values ranging from -24.48 to

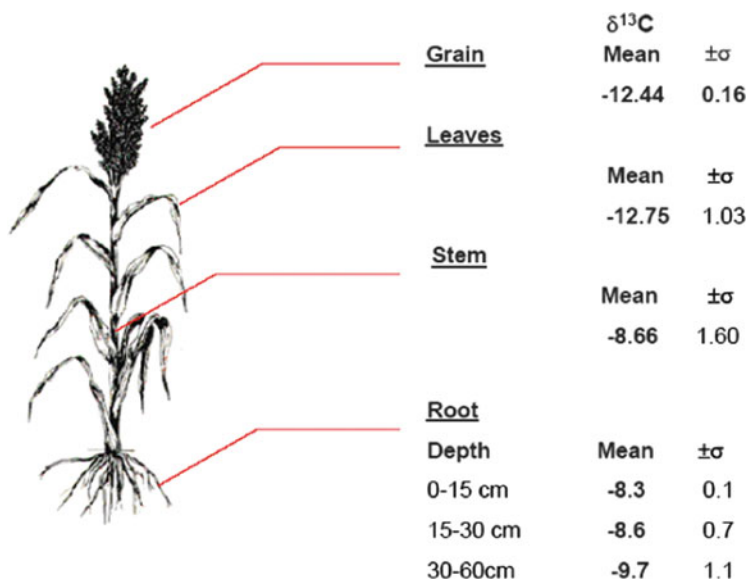


Fig. 5.2 Distribution of the carbon isotopic signature in sorghum plants

Table 5.8 Distribution of the $\delta^{13}\text{C}$ (%) isotopic signature in the soil profile

Soil layer (cm)	$\delta^{13}\text{C}$ organic carbon		$\delta^{13}\text{C}$ total carbon	
	Mean	$\pm\sigma$	Mean	$\pm\sigma$
0–15	–26.89	1.63	–23.01	0.66
15–30	–26.95	1.49	–23.18	0.60
30–60	–26.65	1.59	–22.83	0.97

–20.14%. As for total carbon, we observed a low $\delta^{13}\text{C}$ variability in the layer and within the soil profile (Table 5.8). The uniformity of isotope signature represents an important advantage in field experiments, since even small variations in time can be detected in the isotopic signature of SOM. Average values of organic $\delta^{13}\text{C}$ found at the beginning of the experiment are typical of soils, where C_3 plants have prevalently grown in the past (Boutton et al. 1998).

5.7.4.3 Variation in $\delta^{13}\text{C}$ Values of Soil Organic Carbon

Treatment differences in isotopic signature of SOC after three experimentation years are shown in Fig. 5.3. Generally, our results indicated that $\delta^{13}\text{C}$ values of SOC were affected by the input of both new C_4 -sorghum material and applied compost. This holds true for all treatments (CPT1, CPT2, TRA and 0-N), which were more significantly enriched in ^{13}C than the initial SOC at all soil depths. As expected, variations were larger for plots where no organic matter was applied, except for

sorghum residues (TRA and 0-N) which were more enriched in ^{13}C than plots amended with compost, and especially for the CPT2 double-dose plot. The latter treatment showed a dilution of the root effect in the isotope ratio of SOC. This was due to the large amount of organic carbon applied with compost and characterized by a $\delta^{13}\text{C}$ value of -16% , and it was found to be true at all soil depths. However, the effects of compost application on the soil isotopic signature need to be discussed in more complex terms since a stimulating effect of compost on root production has also been found (data not shown).

The surface $\delta^{13}\text{C}$ of SOC showed the maximum difference between initial SOC (for TRA and 0-N of 4.02% , 4.01% , respectively) (Fig. 5.4).

The roots subsoil deposition was examined through the isotopic signature of soil organic carbon. Figure 5.3 shows the time-frame of the isotopic signature in the TRA treatment (mineral fertilization), where ^{13}C values of SOC were affected by only the input of new C_4 -sorghum residues. Results are related to the experiment start and to the 3 year crop cycles. Overall, the data indicate that after each year SOC was more ^{13}C -enriched than for the experiment start at all sampling depths. A significant increase was recorded after the first and second years. Liang et al. (2002) reported that SOC $\delta^{13}\text{C}$ values during one cycle of maize growth in pots varied from -27.2 to -25.4% and explained the net signature increase (1.8%) by the input of roots and root exudates. These authors found that C_4 -derived SOC during maize growth varied from 1.3 to 12.3% , which accounted for 1.3 – 14.5 g C pot^{-1} . After the first year of sorghum cultivation, our results were very similar to those of Liang et al. (2002) (Fig. 5.3). Conversely, stabilization around the value of -23% was observed in the third year.

Figure 5.4 highlights the effects of 3 years of sorghum cropping on the isotopic signature of organic carbon in the TRA (mineral) and 0-N plots. The mineral fertilizer treatment (TRA) shows more ^{13}C -SOC enrichment than the 0-N treatment. If we compare this finding with the root biomass data reported above, we find

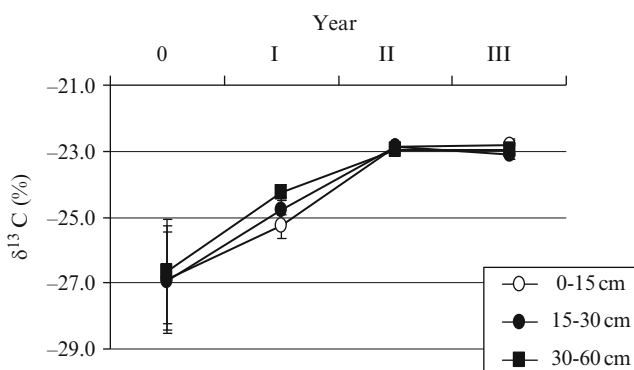


Fig. 5.3 Average $\delta^{13}\text{C}$ (‰) values in the three top layers of the TRA treatment from the beginning of the experiment (0) throughout the end of the sorghum cycle in September 2009 (III). Bars indicate standard deviation of four replicates

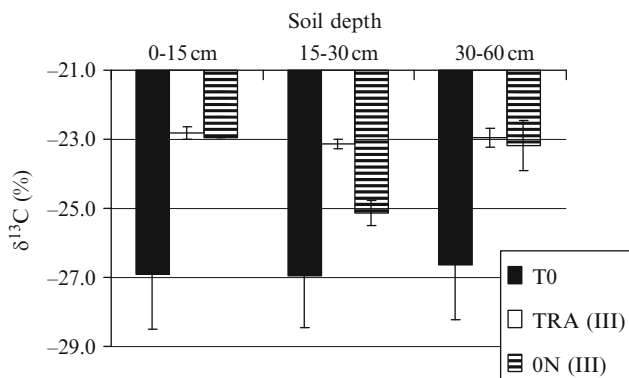


Fig. 5.4 Average $\delta^{13}\text{C}$ (‰) values before the experiment start (T0) and at the end of the sorghum cycle in September 2009 in three soil depths in plot without compost application and in 0-N plot (vertical bars represent standard deviations)

that the larger effect of TRA on soil isotope signature corresponds to a lower root biomass (data not shown). This may indicate that in our experiment the large N supply provided in TRA did not increase total root production but corresponded to a greater fine-root production and turnover (Nadelhoffer 2000). Even a small net root biomass would be compatible with a large root input to SOC and result in a greater ^{13}C signature in TRA than in 0-N.

Figures 5.5 and 5.6 show the profile distribution of $\delta^{13}\text{C}$ as a function of time in the TRA and 0-N treatments. The $\delta^{13}\text{C}$ values show that the modification of the isotope ratio due to root deposition in soil was limited to the top 1 m in the first year, and then gradually extended to deeper soil horizons. In TRA, the effect was slower especially in deep layers, where the top layers' $\delta^{13}\text{C}$ values were reached after 2 years, whereas they were below 1.2 m only in the third year.

Therefore, it only took 1 year of C_4 species cropping to significantly affect the isotopic signature of SOC in the ploughed layer and immediately below that. Moreover, it took only from 2–3 years to ^{13}C -enrich the soil profile down to 210 cm to reach the same values as those found at soil surface where root density is much larger.

The $\delta^{13}\text{C}$ values of vegetation are the major factor in controlling the isotopic signature of soil organic carbon (Boutton 1996). In fact, plant-derived organic carbon is delivered to soil by either roots exudation and its metabolic bioproducts, or dead plant residues (Yoneyama et al. 2006), and both inputs to SOC affect soil isotopic signature. Furthermore, plant-derived organic carbon and soil microbial metabolites are further ^{13}C -enriched by approximately 1‰ as a result of isotopic fractionation during SOM mineralization (CO_2 release) (Boutton 1996), though this effect is negligible in short-term experiments (Shang and Tiessen 2000). In the case of soil ^{13}C -enrichment by dead plant residues, it has been found that roots influence by up to 2‰, as compared to the plant leaf $\delta^{13}\text{C}$ values (Yoneyama et al. 2006). Although no much information is available on the amount of persistent

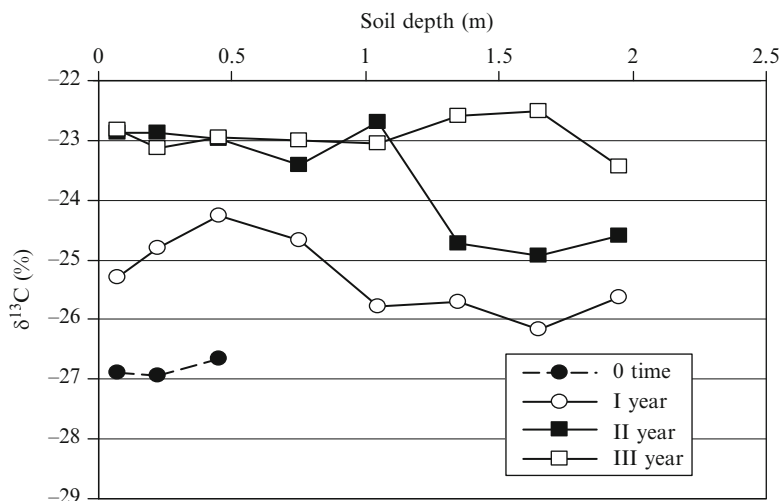


Fig. 5.5 Average $\delta^{13}\text{C}$ (‰) values in the soil profile of the TRA treatment plot down to 2.10 m depth before the experiment start and throughout the three following years of the sorghum cycle

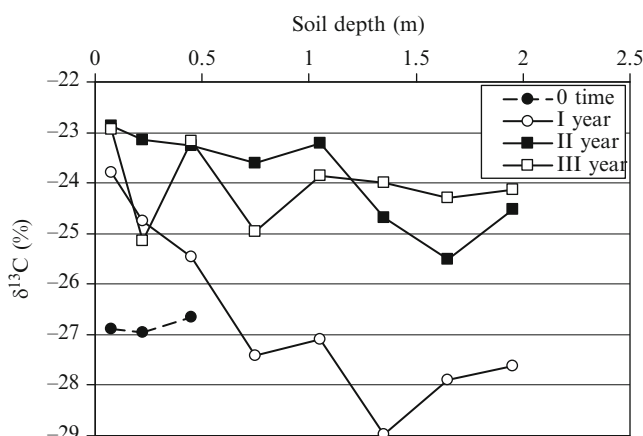


Fig. 5.6 Average $\delta^{13}\text{C}$ (‰) values in the soil profile of the 0-N treatment plot down to 2.10 m depth before the experiment start and throughout the three following years of the sorghum cycle

sorghum roots in organic matter at soil depths, these are more ^{13}C enriched (Fig. 5.2) than aboveground sorghum tissues (Vonfischer and Tieszen 1995; Hobbie and Werner 2004). Moreover, root turnover is likely to contribute to the increase of isotopic signatures in deeper soil layers, especially in coarsely textured soils where rooting is characteristically deep (Schenk and Jackson 2005).

The fraction of soil C derived from new sorghum residues (f_{new}) calculated with the isotopic mixing model (Leavitt et al. 1994) is presented in Table 5.7. One year

after shifting from the C₃ vegetation to the sorghum cultivation (2007), the proportion of sorghum-derived carbon was between 8.24% and 21.29%, while 27.95% was reached after 2 and 3 years. At the end of 2008 and 2009 crop cycles, these percentages were slightly larger for TRA than for 0-N. The sorghum-derived carbon in the overall SOC decreased with depth, and more rapidly below 30 cm. These results agreed with previous works that indicated that C₄-derived SOC was evenly distributed in the upper 30 cm (Angers et al. 1995).

However, our experimental results are difficult to compare with most field data in literature since they refer to a short-time span (3 years). In comparison with literature findings, the percent enrichment found in our experiments was generally large, though it may be due to the low soil organic carbon content of our plots soil.

With regard to longer-time experiments, Gregorich et al. (1995) estimated that 25–35% of maize-derived carbon contributed to total organic carbon in the A_p horizon of a clay loamy soil after 25 years of continuous maize cropping in Canada. Flessa et al. (2000) in Germany found that only 15% of total carbon content in a loamy sandy A_p horizon was maize-derived after 37 years of maize cultivation. Liang et al. (2002) reported that C₄-derived SOC during maize growth varied from 1.3 to 12.3%, accounting for 1.3–14.5 g C pot⁻¹. In France, silty loamy maize soils contained 44% of maize-derived carbon after only 23 years of cultivation (Puget et al. 1995).

It is estimated that 10–40% of C fixed by photosynthesis by arable crops may be lost by roots respiration or released to soil by rhizodeposition (Martin and Merckx 1992), being such large variation in estimates dependent on plant species, growth stage, nutrient status and other environmental conditions. However, other factors affecting the contribution of maize-derived C to SOC is (1) the time elapsed from the C₃ vegetation shift to maize cultivation; (2) removal of crop residues instead of their use in filed mulching. With respect to literature, aboveground biomass was removed for silage use by Flessa et al. (2000) as we did in our experiments, whereas Gregorich et al. (1996) left residues in the field. Nevertheless, our ¹³C-enrichment results are in the same order of magnitude as those reported in the literature.

5.8 Conclusions and Recommendations

Our results from field experiments within the MESCOSAGR project, suggest some recommendations for the management of agro-systems aiming to improve C and N sequestration in soil and efficiency of nutrients uptake. It was shown that compost amendments may be a good strategy to increase N sequestration in soils. In particular, compost-derived nitrogen that was fixed in soil due to repeated amendments was found to gradually decrease, thus changing the ¹⁵N signature mostly toward a reduction in soil macro-aggregates and an increase in fine soil particles. These findings suggest that an evaluation of soil macro-aggregate percentage may become a suitable means to assess short-term changes in agro-ecosystems. Conversely, long-term changes may be usefully monitored by

measuring the content of micro-aggregates, which showed lesser sensitivity to yearly variation of compost quality.

In the first experimentation year, it was found that the OM mineralization rate depended on compost maturity and composition. This implies that it would be possible to regulate nitrogen availability for plants by modifying the quality and composition of compost. On the other hand, in the first year, the contribution of compost to plant nutrition was about 20% of the applied N, while this percentage decreased progressively in the following two experimentation years. Thus, it appears plausible to recommend that a steady N availability to plants over the years should be attained with a yearly supply compost that accounts for the different mineralization rate in diverse years.

Furthermore, we showed that carbon coming from sorghum residues reached about 28% of soil C after 3 years. Sorghum roots turnover contributed also in silt clay soils to increase the carbon isotopic signatures in deeper soil layers, most probably because of the sorghum deep root system. This finding suggests that the inclusion of sorghum in crop rotations may considerably increase carbon sequestration in the whole soil profile and be beneficial to the overall soil quality.

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Chapter 6

Impact of Innovative Agricultural Practices of Carbon Sequestration on Soil Microbial Community

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Abstract This chapter deals with the impact on soil microbiology of innovative management techniques for enhancing carbon sequestration. Within the MESCOSAGR project, the effect of different field treatments was investigated at three experimental sites differing in pedo-climatic characteristics. Several microbiological parameters were evaluated to describe the composition of soil microbial communities involved in the carbon cycle, as well as to assess microbial biomass and activity. Results indicated that both compost and catalyst amendments to field soils under maize or wheat affected microbial dynamics and activities, though without being harmful to microbial communities.

6.1 Microorganisms in Soil

A huge number of microorganisms reside in soil and exert a variety of functions which contribute to ecosystem-level processes and maintenance of primary productivity in terrestrial ecosystems. Growth and metabolism of soil microbes can alter the solubility of soil mineral components and modify soil structure. Moreover, microbes are able to degrade organic compounds and release nutrients, thus regulating nutrients cycling and availability to plants. Microbial activity is responsible for most of soil respiration, thus including oxygen consumption and CO₂ emission, and for immobilization of nutrients in soil microbial biomass. Soil microbes contribute to processes

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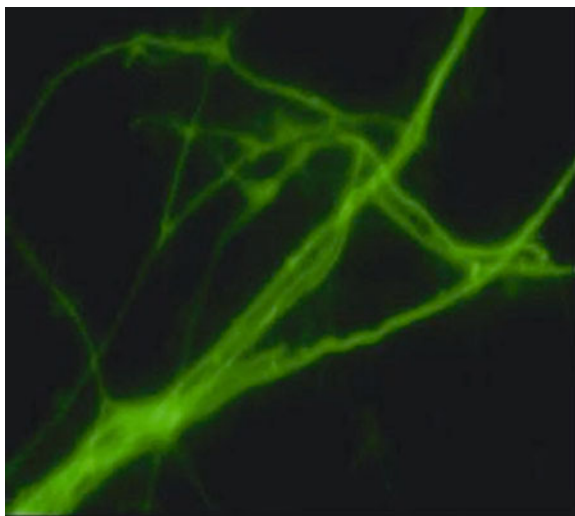
of carbon sequestration in the soil humic fraction since they transform dead organic matter in such a recalcitrant pool. Furthermore, microbial activity is responsible for other essential biological processes, among which is nitrogen fixation. In the absence of soil microbial life, all biochemical transformation cease and the ecosystem sustainability is endangered (Wani and Lee 1995).

Although microbial C in natural soil does not exceed 1–2% of the total soil C (Paul and Clark 1989), it is constituted by a huge variety of organisms whose taxonomy and diversity are poorly known in comparison to aboveground organisms (Barot et al. 2007). Soil microbial communities are extraordinary complex and have been estimated to contain more than 4,000 different genomic equivalent in a single gram of soil (Torsvik et al. 1990). However, microbial species in soil are poorly abundant, most likely because conditions for their survival and growth are limited to a few sites where specific environmental factors, physical–chemical characteristics, and nutrient availability occur. Soil is a very heterogeneous environment encompassing solid, gaseous, and liquid phases. Microbial processes take place at the scale of soil aggregate, which is essentially a porous structure that varies both spatially and temporally. Because soil organic matter located within soil aggregates is physically protected from biodegradation, aggregates enhance carbon sequestration and soil structural stability (Six et al. 2000). Microbial dynamics is influenced by soil structure and the pore-size distribution within soil aggregates. Bacteria are restricted to grow and feed on the exposed surfaces of organic matter and/or inorganic particles. Fungi penetrate large pieces of organic matter and can thus extend their hyphae for centimeters and even meters in soil. The location of bacteria and fungi influences their activity as well as their survival to predation. The larger size of fungi may make them more vulnerable to predation, whilst small pores provide refuge for bacteria against predators (Six et al. 2006).

Plants are responsible for a large input of organic carbon into soil, thus becoming the main determinant of microbial life in soil through the complex food web of debris. It has been found that the type of aboveground plant community influences the composition of belowground soil microbial community in natural ecosystems (Reynolds et al. 1997; Côté et al. 2000; Smolander and Kitunen 2002; Rutigliano et al. 2004), as well as in semi-natural grasslands (Singh et al. 2009) and in agro-ecosystems (Marschner et al. 2001; Hedlund 2002). Moreover, arbuscular mycorrhizal fungi require a plant host to survive.

Consequently, plants influence the spatial distribution of bacteria and fungi in soil (Kirk et al. 2004). The site of greatest soil activity is the root–soil interface, or rhizosphere. Roots affect soil structure, aeration, and biological activity and deeply impact soil microbial communities in their immediate vicinity, greatly increasing population densities of bacteria and fungi (Buyer et al. 2002; Marschner et al. 2002). As plants may allocate up to 40% of the assimilated carbon belowground, roots are the major source of organic matter into the surrounding soil through both root debris and exudates. Exudates are made up of sugars (50–70%), carboxylic acids (20–30%), and amino acids (10–20%), i.e., carbon-rich substrates that are able to regulate decomposition of recalcitrant soil organic carbon by controlling the activity and relative abundance of fungi and bacteria (Cheng et al. 2003; de Graaf et al. 2010).

Fig. 6.1 Living fungal hyphae observed by fluorescence microscopy after treatment with the viability stain fluorescein diacetate (FDA)



Among soil organisms, actinomycetes, fungi (Fig. 6.1), and bacteria are the most abundant and most metabolically active. Bacteria and fungi generally comprise >90% of the total soil microbial biomass and are responsible for most of soil organic matter decomposition (Six et al. 2006). Fungi incorporate more soil C in their biomass than bacteria, and fungal cell walls are more recalcitrant than bacterial cell walls. Therefore, carbon sequestration may be larger in soils dominated by fungal communities than in those whose communities are dominated by bacteria (Six et al. 2006). Moreover, actinomycetes, fungi, and bacteria include organisms (such as aerobic and anaerobic cellulolytic bacteria), which are able to degrade cellulose and lignin (McCarthy and Williams 1992; Wellington and Toth 1994; Berg and McLaugherty 2008). In fact, degradation of plant biopolymers is the fundamental step in the carbon cycle and this process is important in soil systems. Since plants are the most relevant carbon providers in soil and cellulose and lignin are the most abundant constituents of plant tissues, they consequently represent the largest source of carbon in soil. Microorganisms transform plant polymers into simpler compounds, which are then made available to other microbial populations, and/or are stabilized in humic substances. The mineralization process during metabolic consumption of polymer by-products ultimately produces carbon dioxide that is emitted to the atmosphere. Moreover, actinomycetes regulate the microbial equilibrium in soil through production of antibiotics and probiotics that stimulate microflora and plant growth.

Fungi play a central role in many soil microbiological processes thus influencing the structure and functioning of plant communities and soil ecosystems. Fungi are immensely diversified, both structurally and functionally, and adopt different trophic strategies, since they occur as saprotrophs, symbionts, and pathogens. Individual fungi can often simultaneously colonize different substrates, such as living or dead plant tissues, woody debris, soil animals, and mineral substrates, thus

allowing the transfer of substances. Filamentous fungi are responsible for decomposition of organic matter (e.g., lignin degradation) and nutrient cycling (Parkinson 1994; Van Elsas et al. 2007) and their activity is critical in regulating the availability of nutrients for plant growth. Moreover, fungi are food for nematodes, mites, and other larger soil organisms, which are also predators or parasites of other soil organisms.

6.2 Impact of Agricultural Management on Soil Microbial Communities

Agricultural management produces a disturbance of both abiotic and biotic components of soils. The most negative impact is the loss of soil organic matter (SOM) (Balesdent et al. 1999), with consequent increase in soil erosion and decrease in soil structure stability (Bronick and Lai 2005) and fertility. In agro-ecosystems, soils degradation is the outcome of unsustainable techniques aimed to increase production in the short term without paying attention to the conservation of soil resources. Agricultural land management, such as cropping systems (Kuske et al. 2002) and tillage systems (Peixoto et al. 2006) may affect soil characteristics, including physical, chemical, and biological properties and processes. It has been observed that tillage reduces soil microbial populations (Ibekwe et al. 2002) and different enzymatic activities (Carpenter-Boggs et al. 2003). Tillage has a catastrophic effect on fungi as it physically breaks the hyphae and severely damages the mycelium, thus consequently hampering the stability of soil aggregates whose particles are transiently bound together by fungal hyphae. Six et al. (2006) showed that no-tillage enhances fungal biomass with a consequent quantitative and qualitative SOM improvement that is attributed to the positive influence of fungi on aggregate stabilization.

Alternative agricultural techniques, such as minimum tillage, have been developed to improve soil quality by progressively recovering soil organic matter (Lu et al. 2000). In long-term experiments on tillage comparison along two climatic gradients, Frey et al. (1999) observed that in response to reduced tillage both fungal biomass and fungal/bacterial biomass increased at all sites. Thus, less intensively managed agro-ecosystems, such as those managed with no-tillage practices, more closely resemble natural ecosystems, which are dominated by fungi (Bayley et al. 2002). On the other hand, intensive cultivation leads to progressive SOM depletion with a consequent microbial biomass reduction, loss of microbial diversity and reduction of microbial activities (Bastida et al. 2006). Buckley and Schmidt (2001) performed a large-scale experiment with replicated plots under distinct management regimes ranging from conventionally tilled annual cropping systems to abandoned fields. The effects of tillage, fertilization, and plant community composition on the structure of microbial community were evaluated. They found that microbial communities differed significantly between fields that had never been cultivated and those with a long-term history of cultivation. However, microbial community structure was very similar in plots that shared a long-term history of

cultivation, despite differences in plant community composition, chemical inputs, tillage, and productivity. They argued that microbial communities respond to soil characteristics which require long time periods to recover from disturbance. Indeed, the organic pools of carbon and nitrogen can be depleted by long-term agricultural practices and may require decades or even centuries to recover pre-agricultural levels. In a study dealing with soil quality as related to different land uses in Southern Italy, Marzaioli et al. (2010) report that soil quality, evaluated by a set of parameters including microbial indexes, was strongly and negatively affected by permanent crop management. Moderate grazing activity, as well as crop management comprising mulch cover on soil, had a lower negative impact. Moreover, these authors found that the abandonment of cultivated lands, with consequent development of shrublands, produced an improvement of soil quality, thus suggesting a good recovery capacity.

Microbes are also affected by fertilization (Marschner et al. 2003), both directly and indirectly. Zhong and Cai (2007) showed that the long-term application of P and N indirectly affected microbial parameters in soil by increasing crop yields and promoting SOM accumulation. Fertilizers used in agricultural production systems include mineral (urea, ammonium nitrate, sulfates, and phosphates) and organic (animal manures, biosolids, and composts) fertilizers. Composted materials vary widely in their characteristics such as dry and organic matter content, pH, carbon and nitrogen content, plant residues, and microbial community composition. Application of compost to soil is used to improve soil fertility and structure since it increases the carbon, nitrogen, and phosphorus content in soil (Hartz et al. 2000; Filcheva and Tsadilas 2002; Adediran et al. 2003) and contributes to the stabilization of soil aggregates (Bresson et al. 2001; Barzegar et al. 2002). Although compost amendments differ in origin of material and application rates, organic amendments to soil generally result in an increase of microbial proliferation in soil (Bünemann et al. 2006). In fact, organic-matter-rich amendments are also used to stimulate soil microflora in degraded and arid environments (Ouedraogo et al. 2001; Ros et al. 2003). However, compost amendment can also cause negative effects by altering the microbial biomass, size, function, and diversity, if contaminant residues are present at toxic levels (Gomez 1998; Zheljazkov and Warman 2003). Nevertheless, soil microbial response is generally transient (Calbrix et al. 2007) and microbial characteristics can return to their baseline within a few years (Speir et al. 2003; Garcia Gil et al. 2004) depending on nature of organic amendments and level of compost application (Albiach et al. 2000; Garcia-Gil et al. 2000).

6.3 Microbial Parameters as Indexes of Soil Quality

Because of the fundamental role in mediating soil processes and the responsiveness to soil managements, microbial abundance, diversity, and activity are among the most important soil quality parameters (Andrews and Carroll 2001; Karlen et al. 2001, 2003; Andrews et al. 2003; Anderson and Domsch 2010). In fact, the effect of

agricultural management on microbial community is directly related to changes in soil quality (Schloter et al. 2003), that encompass the size and diversity of specific functional microbial groups (Helgason et al. 1998; Chang et al. 2001).

A great number of methods have been developed to determine the presence and activities of microbial communities in soil. Some of them are internationally standardized (Winding et al. 2005), such as measures of population size for either a single organism type, a functional group, or a whole community. The effect of agricultural managements on soil microorganisms can be measured with changes in both community size (cell number) or microbial biomass, and biological activity, such as soil respiration. However, although addition to soil of good quality compost may increase global microbial biomass and enhance enzyme activity (Albiach et al. 2000; Perucci et al. 2000; Debosz et al. 2002), the specific responses of various bacterial groups to changing environment in agricultural soils are poorly known (Buckley and Schmidt 2001; Kiikkilä et al. 2001; Chander and Joergensen 2002). Moreover, several studies showed that, in order to assess fertilizers' effects, microbial enumeration methods by plate counts (Sarathchandra et al. 1993) and nematode counts (Parfitt et al. 2005) are possibly more sensitive than measurements of microbial biomass.

Fungal and microbial biomass is thought to be a sensitive indicator of soil quality and an early predictor of changes in SOM dynamics. In fact, the rate of microbial fraction turnover is relatively fast (2–6 years) as compared to more than 20 years of SOM turnover (Jenkinson 1990). Thus, fungal and microbial biomass are SOM living components (Jenkinson and Ladd 1981) representing an active soil carbon that is more sensitive to soil management than total organic carbon (Frey et al. 1999; Bayley et al. 2002; Weil and Magdoff 2004; Six et al. 2006). However, microbial biomass C generally reflects the amount of total organic matter content. Both SOM and microbial biomass decline under agricultural or land disturbance, indicating exploitation of organic resources and impact of differing tillage systems, fertilizers, and crop rotations (Luizao et al. 1992; Sparling 1997; Frey et al. 1999; Vineela et al. 2008). Soil respiration is the best indicator of the whole metabolic activity of soil microorganisms, since it allows comparison of different soils and soil management effects (Machulla 2003; Solaiman 2007). Soil respiration, as referred to SOM content to give a coefficient of organic matter mineralization (CEM), may express the potential capacity of soil to accumulate or mineralize carbon (Diaz-Raviña et al. 1988).

6.4 Impact of Different Agricultural Practices on Soil Microbial Communities: The Mescosagr Case Study

Within the National project MESCOSAGR, we investigated the impact on soil microorganisms of two innovative technologies applied to sequester carbon in agricultural soils. The hypothesis was that the structure and activity of microbial

communities would be influenced by (1) addition of compost, a humified and hydrophobic material that protects the easily degradable organic fraction, and (2) in situ photo-oxidative polymerization of native SOM under the action of a biomimetic catalyst (CAT) (iron–porphyrin). The effect of these two technologies on soil microorganisms was compared with that exerted by traditional deep tillage and minimum tillage. The latter is an agronomic practice commonly used to reduce SOM depletion and limit CO₂ emission from soil into the atmosphere (see Chap. 3).

Two types of approaches were pursued to estimate soil microbiological parameters (1) one aimed to characterize the composition of soil microbial communities involved into carbon cycle, (2) another one based on a holistic view that considers the microbial biomass as a whole, without distinguishing its individual components. The first approach is based on the Surface Spread Plate Count Method and Pour Plate Method, which use selective media to identify the major microbial groups involved in the different steps of organic matter decomposition, i.e., total aerobic heterotrophic bacteria, cellulolytic bacteria, fungi, and actinomycetes. This approach allows determining the size and composition of microbial communities and has been used to assess changes in the soil biota in response to land management, thereby providing an indicator of soil biological status (Harris and Birch 1992). The second approach is based on the determination of microbial community characteristics which include abundance and activity (1) abundance of fungal cells is measured by fluorescence microscopy; (2) soil microbial biomass is determined as microbial C by the SIR (Substrate Induced Respiration) method; (3) microbial respiration per unit of organic C becomes a coefficient of endogenous SOM mineralization. The combination of these two experimental approaches is expected to provide most information regarding the effects of soil managements on microbial communities.

6.5 Soil Sampling and Microbiological Analyses

The study comprised three different sites (Napoli, Torino, and Piacenza) under different soil and climate conditions. All microbial parameters were assessed in both the bulk soil and the rhizo soil. Soil samples were collected from the experimental plots under either maize or wheat (see Chaps. 3 and 7 for details on the field trials) during three consecutive years (2006–2007–2008). Bulk-soil samples were collected from the 0–15 cm soil layer after maize harvest (September) and before wheat sowing (November). Rhizo-soil samples were collected during stem elongation (April–May for wheat rhizosphere, and July for maize rhizosphere). Bulk-soil samples were a mix of three subsamples collected in three different locations for each treatment plot. Rhizosphere samples consisted of soil adhering to total roots of three crop plants collected from each treatment plot. The roots were shaken vigorously to separate the rhizo soil. All samples were collected in triplicate, brought to laboratories, stored in polyethylene bags at 4°C for no more than 24–48 h before soil microbiological analyses were conducted.

6.5.1 Microbial Counts

Microbial counts were performed according to Italian official methods (Picci and Nannipieri 2003). Briefly, soil samples (10 g) were shaken for 30 min in 90 ml of physiological solution containing 0.162 g of tetrasodium pyrophosphate to detach the bacteria from soil particles. After soil particles were allowed to settle for 15 min, the solution was diluted tenfold in a series. Selected populations of soil microbial community were detected at 28°C by using the Surface Spread Plate Count Method (aerobic bacteria) and the Pour Method (anaerobic bacteria). Three plates were used per each dilution. Total heterotrophic aerobic bacteria were counted in Plate Count Agar (Oxoid Ltd., Oxford, UK). The plates were incubated for 3 days.

Mould and yeast were cultivated on Malt Agar (Oxoid Ltd., Oxford, UK) supplied with chloramphenicol (100 mg L⁻¹) for 3 days (Allievi and Quaroni 2003).

For the isolation of actinomycetes, Starch-Casein Agar (10 g soluble starch, 0.30 g casein, 2 g KNO₃, 2 g NaCl, 2 g K₂HPO₄, 0.01 g FeSO₄, 0.05 g MgSO₄, 0.02 g CaCO₃, 1,000 ml distilled water, 17 g bacteriological agar, pH 7.0) was used (Kuster and Williams 1964). The medium also contained cycloheximide at 100 µg ml⁻¹ to minimize fungal contamination. The plates were incubated for 14 days.

The medium used for aerobic and anaerobic cellulolytic bacteria was composed by 5 g L⁻¹ carboxymethylcellulose (CMC) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 1 g L⁻¹ (NH₄)NO₃, 1 g L⁻¹ yeast extract, 50 ml L⁻¹ standard salt solution, 1 ml L⁻¹ trace elements solution, 15 g L⁻¹ bacteriological agar, at pH 7.0. The plates, incubated in aerobic or anaerobic (Oxoid's Anaerogen™ System) (Allievi and Möller 1992) conditions for 7 days, were stained with Congo red (0.1%) for 20 min and bleached with NaCl (5 M) for 20 min to put in evidence cellulolytic activities by developing clear haloes around the colonies (Kluepfel 1988). All microbial counts were carried out in triplicate and microbiological data were expressed as CFU g⁻¹ of dry soil.

6.5.2 Active Fungal Mycelium

Metabolically active hyphae were estimated by fluorescence microscopy. Soil samples were sieved through a 2-mm mesh, suspended in a solution (1 g of fresh soil in 100 ml) of phosphate buffer (60 mM, pH 7.5), and homogenized at 6,000 rpm for 2 min. 0.5 ml of suspension were collected and filtered under vacuum on nitrocellulose filter with a pore size of 0.45 µm. The sample was treated with fluorescein diacetate (FDA) (Söderström 1977, 1979). This stain penetrates rapidly in cells and is hydrolyzed to fluorescein by different enzymes such as protease, lipase, and esterase. After clearing by immersion oil, the preparations for active

mycelia were observed at a magnification of $400\times$ and 20 microscopic fields were counted. Active mycelia were estimated by the intersection method (Olson 1950) and their mass calculated on the basis of an average hyphae cross section of $9.3 \times 10^{-6} \text{ mm}^2$, a density of 1.1 g ml^{-1} and a dry mass of 15% of wet mass (Berg and Söderström 1979). The fungal biomass was expressed as mg of fungal biomass per gram of soil dry weight.

6.5.3 Microbial Biomass

Microbial biomass (C_{mic}) was determined by the SIR method (Anderson and Domsch 1978) that is based on the measurement of CO_2 evolution from soil in response to addition of glucose, an easily mineralizable substrate. The magnitude of the respiratory response, as measured after incubation under controlled temperature and humidity conditions, is related to the amount of active biomass in the soil sample, and can be converted to mg of microbial biomass carbon using a conversion factor introduced by Sparling (1995):

$$C_{\text{mic}}(\text{mg C g}^{-1} \text{d.w.}) = 50.4 \times \text{respiration rate (ml CO}_2 \text{ g}^{-1} \text{d.w.h}^{-1})$$

Microbial biomass C was measured by mixing in 30 ml vials 1 g of each soil sample (sieved through a 2-mm mesh) with 2 ml of 75 mM D-glucose (27.3 mg g^{-1} soil d.w.). The vials were then sealed tightly and incubated for 4 h in the dark at 25°C . The evolution of CO_2 was measured by gas chromatography (Fisons GC 8000 series). The CO_2 values were corrected for the CO_2 measured in a blanc vial containing only the soil sample and 2 ml of water, and were reported as mg of microbial carbon.

6.5.4 Microbial Activity

Soil microbial activity can be estimated by measuring CO_2 respired from soil, as a well-established parameter to monitor SOM decomposition (Anderson 1982). Soil respiration is highly variable and its natural fluctuation depends on substrate availability, moisture, and temperature (Alvarez et al. 1995; Brookes 1995). For valid comparisons among soils, respiration measurements must be conducted under controlled laboratory conditions (Anderson 1982). Here, the basal respiration of soil samples was estimated by gas chromatography (Fisons GC 8000 series) as CO_2 evolution in standard conditions (4 h of incubation at 25°C , at dark), after adding 2 ml distilled water to 1 g of soil (Degens et al. 2000). The basal respiration was expressed in $\mu\text{g CO}_2$ evolved per gram soil per unit of time. Therefore, the rate of OM mineralization and, hence, the potential capacity of soil to accumulate or

dissipate carbon, is comprised in the coefficient of endogenous mineralization (CEM) that was calculated from soil respiration and SOM content, whereby CEM represents the CO₂ evolved from soil per unit of organic C.

6.5.5 Statistical Analyses

To assess the differences among the project treatments as well as among years in each experimental site, data for cultivable microbial populations were analyzed by using XLSTAT-6.1 and applying standard analyses of variance (one-way and two-way ANOVA) at $p \leq 0.05$ level.

The three-way ANOVA followed by Holm–Sidak post hoc test for pair-wise comparison of means (at $p \leq 0.05$ level) was used to elaborate data of active fungal mycelium (AFM), microbial biomass and microbial respiration and to assess the differences among treatments and experimental sites, as well as those between bulk soil and rhizo soil. A two-way ANOVA was performed for CEM since only bulk-soil values for organic carbon were available due to missing measurements of SOM in the rhizosphere. Statistical analyses were performed by using Sigma-Stat-3.1 for Windows software package.

6.6 Effects of Compost Amendments

Soil managements, such as traditional and minimum tillage, induce reduction of SOM content and decrease of soil structural stability and, thus, have a great impact on functional processes of soil microbial communities. Application of materials rich in organic matter, such as compost, may be used to recover and/or improve soil structure and fertility. Amendments with compost can also strongly influence and modify the size, biodiversity, and activity of the microbial communities in soil (Albiach et al. 2000). Since compost is a source of nutrients which can be used by microorganisms, compost addition usually increases soil microbial biomass and global activity (Bailey and Lazarovits 2003).

6.6.1 Microbial Counts

The effect of compost amendment (COM-2) on the biomass of cultivable communities, as compared to traditional (TRA) and minimum (MIN) tillage, was studied in three different experimental sites (Napoli, Torino, and Piacenza) of the MESCOSAGR project during 3 years (2006, 2007, and 2008).

Microbial populations were significantly affected by agronomic practices. In fact, in all three sites the microbial populations were drastically reduced after

3 years of experimentation in both bulk soil and rhizo soil. This trend was most marked in field plots at Napoli, in which all enumerated microbial populations in COM-2 soils were 1 Log CFU g⁻¹ smaller than those found in TRA and MIN soils. In particular, the COM-2 bulk soil showed a negative cumulated effect on total heterotrophic aerobic bacteria, fungi, actinomycetes, and aerobic and anaerobic cellulolytic bacteria due to repeated compost applications to soil. In fact, the

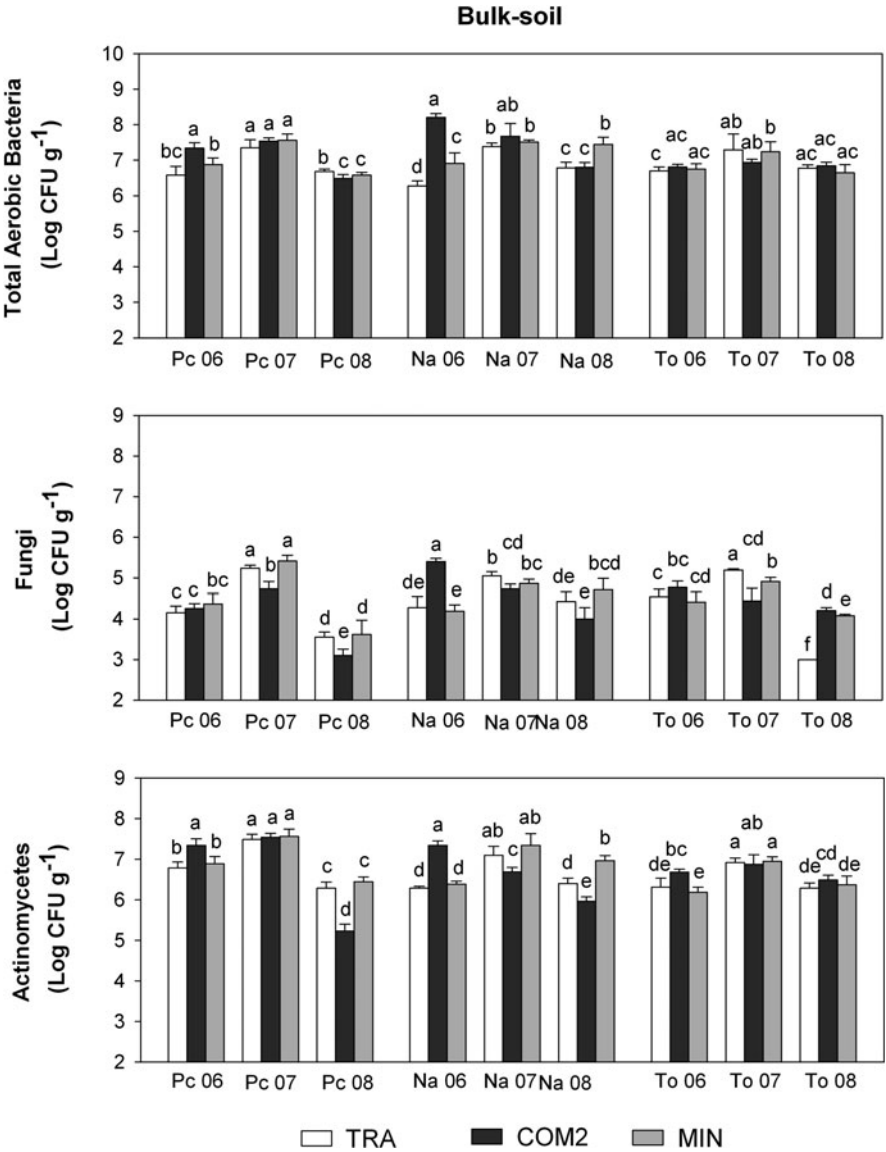


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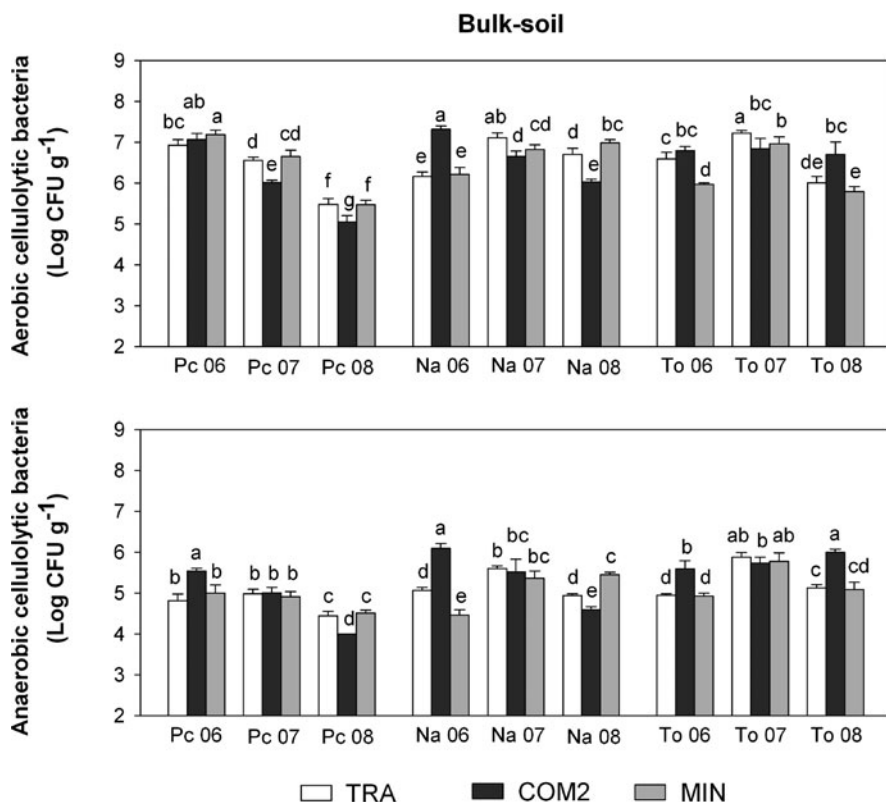


Fig. 6.2 Effect of management practices (*TRA* traditional amendment, *MIN* minimum tillage, *COM-2* compost amendment) on (a) total aerobic bacteria, fungi, and actinomycetes (mean of Log CFU g⁻¹ of soil $\pm$ SE) in bulk soils of Piacenza (Pc), Napoli (Na), and Torino (To). Different letters indicate significant difference among treatments and years (ANOVA–Tukey test; $p < 0.05$) within site (b) aerobic and anaerobic cellulolytic bacteria (mean of Log CFU g⁻¹ of soil $\pm$ SE) in bulk soils of Piacenza (Pc), Napoli (Na), and Torino (To). Different letters indicate significant difference among treatments and years (ANOVA–Tukey test; $p < 0.05$) within site

amount of microbial populations after the first compost application (2006) increased significantly more in COM-2 than in TRA and MIN (Fig. 6.2a, b), before declining in the following 2 years (2007–2008). A similar trend was observed in maize rhizo soil at Napoli (Fig. 6.3a, b), whereby a significant abrupt reduction of aerobic (from 8.07 ± 0.08 to 6.21 ± 0.16 Log CFU g⁻¹) and anaerobic (from 6.72 ± 0.12 to 4.93 ± 0.05 Log CFU g⁻¹) cellulolytic bacteria was observed in the third year of compost amendments (Fig. 6.3b).

At the Piacenza site, COM-2 plot was characterized by the lowest microbial values mainly in 2008 year (Fig. 6.2a, b), even though all treatments negatively influenced microbial populations in bulk soil for all 3 years of experimentation. The cumulative negative effect was clearly detectable in aerobic and anaerobic cellulolytic bacteria, which showed a decrease of 1–2 Log cycles in the third treatment

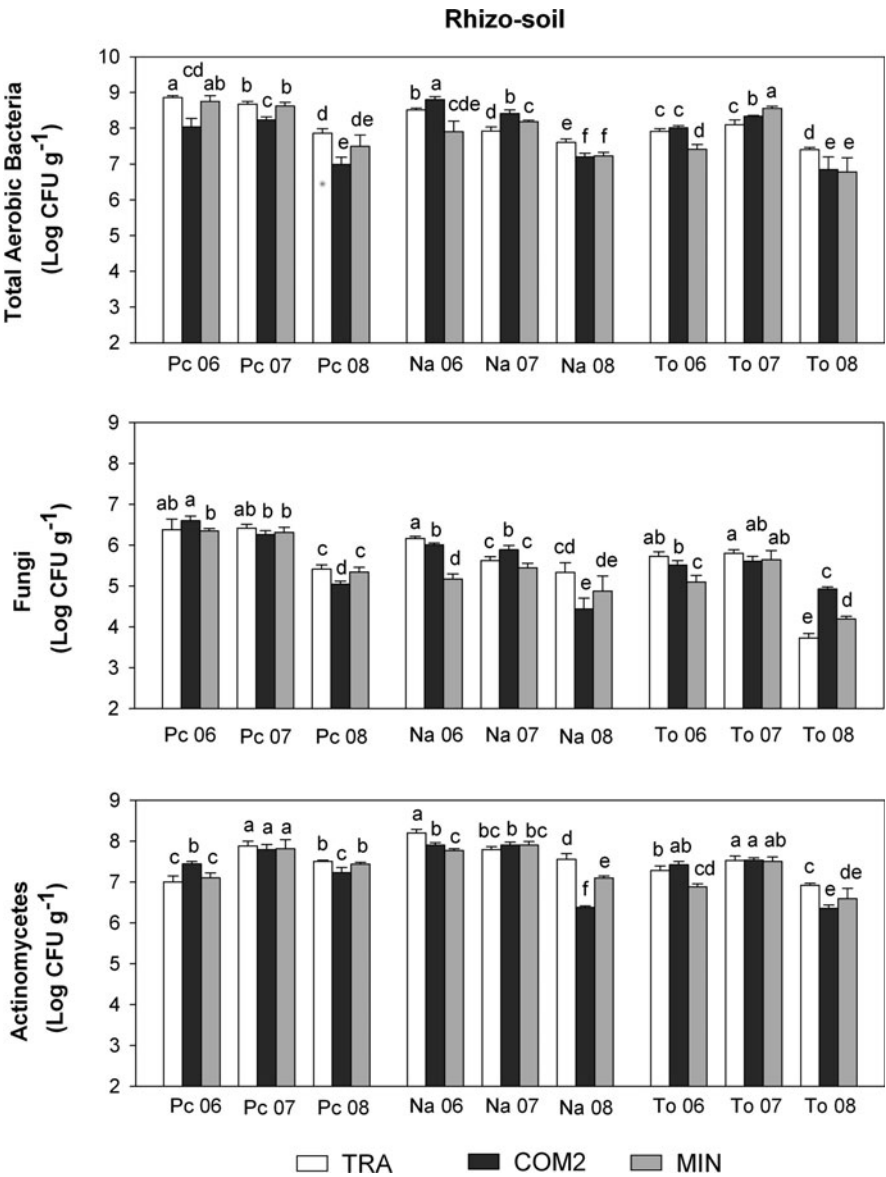


Fig. 6.3 (continued)

year (Fig. 6.2b). The reduction of microbial populations, and particularly of cellulolytic bacteria, is interesting since the cellulose degrading enzymes of these populations are directly involved in key OM decomposition steps. In fact, the reduction of functional group of cellulolytic bacteria may result in an increase of organic matter stabilization due to compost addition. Even if this negative behavior

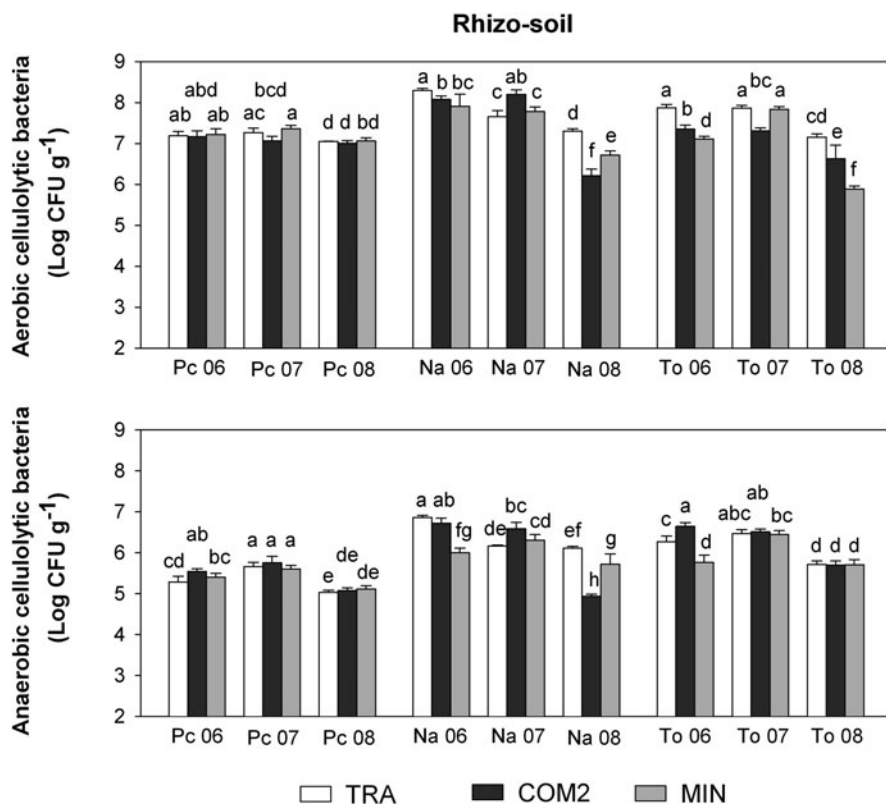


Fig. 6.3 Effect of management practices (*TRA* traditional amendment, *MIN* minimum tillage, *COM-2* compost amendment) on (a) total aerobic bacteria, fungi, and actinomycetes (mean of Log CFU g⁻¹ of soil $\pm$ SE) in rhizo soils of Piacenza (Pc), Napoli (Na), and Torino (To). Different letters indicate significant difference among treatments and years (ANOVA–Tukey test; $p < 0.05$) within site (b) aerobic and anaerobic cellulolytic bacteria (mean of Log CFU g⁻¹ of soil $\pm$ SE) in rhizo soils of Piacenza (Pc), Napoli (Na), and Torino (To). Different letters indicate significant differences among treatments and years (ANOVA–Tukey test; $p < 0.05$) within site

was detected also in the rhizo soil of Napoli, no significant differences among soil management treatments were observed in Piacenza for aerobic and anaerobic cellulolytic bacteria in maize rhizo soil all through the 3 years (Fig. 6.3b). Therefore, the detrimental effect of compost may have been reduced by root exudation in the rhizosphere of maize cropping, as an additional organic carbon source stimulating the microbial growth. The size and composition of rhizosphere microflora is mostly plant-dependent, a phenomenon known as the “rhizosphere effect” (Burr and Caesar 1984) and is attributed to emission of root exudates. Composition of exudates was shown to depend on plant species (Wieland et al. 2001; Singh et al. 2007), as well as on the plant development stage (Jaeger et al. 1999; Yang and Crowley 2000; Feng et al. 2003), environmental conditions, and management practices (Paterson and Sim 1999, 2000).

Results different from those of Napoli and Piacenza were obtained from Torino, where compost application did not show the same cumulative effect on soil microbial communities in either bulk or rhizo soil. In fact, bulk soil from COM-2 did not reveal significant difference among the three experimental years in microbial densities of heterotrophic aerobic bacteria, actinomycetes, and aerobic cellulolytic bacteria. By contrast, in 2008, anaerobic cellulolytic bacteria and fungi slightly increased or decreased, respectively (Fig. 6.2a, b). However, the medium used in this study for fungal population mainly selects for a physiological type of fungi characterized by rapid germination of spores and high rate of mycelial growth. Such fungi, which are pioneer colonizers, are able to use ephemeral substrates readily. Their rapid growth results in a sudden spike of activity followed by a rapid decline, since they are unable to degrade abundant substrates such as the resistant ligno-cellulosic structures present in the green compost of this study.

Overall, microbial populations detected in Torino rhizo soil at the third year of experimentation (2008) showed a significant decrease of $1\text{--}2 \text{ Log CFU g}^{-1}$ for all soil treatments.

The different effect of compost amendment on cultivable microbial biomass at the three field sites should be ascribed to different soil texture and climatic conditions, which are the main determinants of structure and activity of microbial communities. Moreover, the compost used in the experiments was a green waste compost (for chemical composition of the compost see Chaps. 3 and 4). Green waste compost contains both readily decomposable (cellulose) and more recalcitrant (lignin) fractions from plant litter (Standing and Killham 2007). In a short-term experiment, Pérez-Piqueres et al. (2006) evaluated the impact of organic amendments on soil microbial characteristics by using green waste and spent mushroom composts. They found that the microflora in two different soils was influenced by the type of compost. Green waste compost did not modify the densities of cultivable bacteria and fungi in either soil, while the spent mushroom compost significantly increased bacterial and fungal densities in both the clayey and sandy-silty-clay soil, respectively.

Therefore, the cumulated negative effect recorded in Napoli and Piacenza sites (silty-clay-loam soils) may be due to an interaction of compost with the abundant clay particles, which might protect organic matter physically and/or chemically. The mechanism by which organic matter is adsorbed on clay determines its bioaccessibility and the ability of microorganisms to use OM as substrate and to produce extracellular enzymes. Moreover, the presence of chaotropic and antichaotropic ions can influence the nutritional status of microhabitats (Stotzky 1997). By contrast, in Torino bulk soil with a low content of clay (sandy-loam soil), compost amendment led to a significant microflora stimulation, as compared to traditional and minimum tillage.

The largest number of cultivable microorganisms found only in the first experimental year in the bulk soil of COM-2 at all experimental sites should be attributed to the introduction of new community members with the compost rather than to a stimulation of the indigenous community. In fact, both in Napoli and Piacenza, the negative effects on microorganisms were generally observed at the end of the third experimental year.

6.6.2 AFM, Microbial Biomass and Activity

The results of microbial counts were confirmed by evaluating active fungal biomass (AFM), microbial carbon (C_{mic}), and soil respiration. The soils of Torino and Napoli showed statistically significant differences for microbial and fungal biomass and CEM in 2008 and for all investigated parameters in 2007 (Table 6.1). In the third year (2008) of experimentation (Table 6.1), active fungal biomass (AFM) and microbial carbon (C_{mic}) were significantly affected by compost amendment (COM-2). Moreover, when considering the variability within groups, a significant effect was observed for *soil* (bulk/rhizo) and *site* (Napoli/Torino), as well as for

Table 6.1 Levels of significance (p values from ANOVA) for effects of compost amendments on microbial biomass and activity in bulk soil and rhizo soil at Napoli and Torino sites, and differences between years

		AFM	C_{mic}	Respiration		CEM
	dF	p			dF	p
<i>2007 (Three-way)</i>						
Treatments (TRA-COM)	1	0.201	0.524	0.624	Treatments (TRA-COM)	1 0.239
Soil (Bulk–Rhizo)	1	0.164	<0.001	0.039	Site (Napoli–Torino)	1 <0.001
Site (Napoli–Torino)	1	0.002	<0.001	0.001	Treatments $\times$ site	1 0.594
Treatments $\times$ soil	1	0.469	0.550	0.735		
Treatments $\times$ site	1	0.149	0.723	0.850		
Soil $\times$ site	1	0.625	<0.001	0.022		
Treatments $\times$ soil $\times$ site	1	0.611	0.374	0.891		
<i>2008 (Three-way)</i>						
Treatments (TRA-COM-MIN)	2	0.013	0.019	0.068	Treatments (TRA-COM-MIN)	2 0.001
Soil (Bulk–Rhizo)	1	<0.001	<0.001	0.002	Site (Napoli–Torino)	1 <0.001
Site (Napoli–Torino)	1	<0.001	<0.001	0.147	Treatments $\times$ site	2 0.035
Treatments $\times$ soil	2	0.007	0.104	0.405		
Treatments $\times$ site	2	0.687	0.012	0.079		
Soil $\times$ site	1	<0.001	<0.001	<0.001		
Treatments $\times$ soil $\times$ site	2	0.019	0.089	0.091		
<i>2007–2008 (Three-way)</i>						
Years (2007–2008)	1	<0.001	<0.001	<0.001	Years (2007–2008)	1 0.734
Site (Napoli–Torino)	1	0.004	0.084	<0.001	Site (Napoli–Torino)	1 <0.001
Soil (Bulk–Rhizo)	1	<0.001	<0.001	0.001	Years $\times$ site	1 <0.001
Years $\times$ site	1	<0.001	<0.001	<0.001		
Years $\times$ soil	1	<0.001	<0.001	0.095		
Site $\times$ soil	1	<0.001	<0.001	<0.001		
Years $\times$ site $\times$ soil	1	0.001	0.028	0.028		

dF degree of freedom, AFM active fungal mycelium, C_{mic} Microbial carbon, CEM coefficient of endogenous mineralization, TRA conventional tillage, COM compost amendment, MIN minimum tillage. Values in bold are statistically significant

rhizosphere $\times$ *site* interaction (Table 6.1). The interaction *treatment* $\times$ *soil* was significant only for AFM in 2008, while the interaction *treatment* $\times$ *site* was significant only for C_{mic} COM-2 significantly reduced AFM in the Torino bulk soil (Fig. 6.4), while no significant effect was detected in the bulk soil of Napoli (Fig. 6.4). In the maize rhizo soil of both Napoli and Torino, a significant reduction

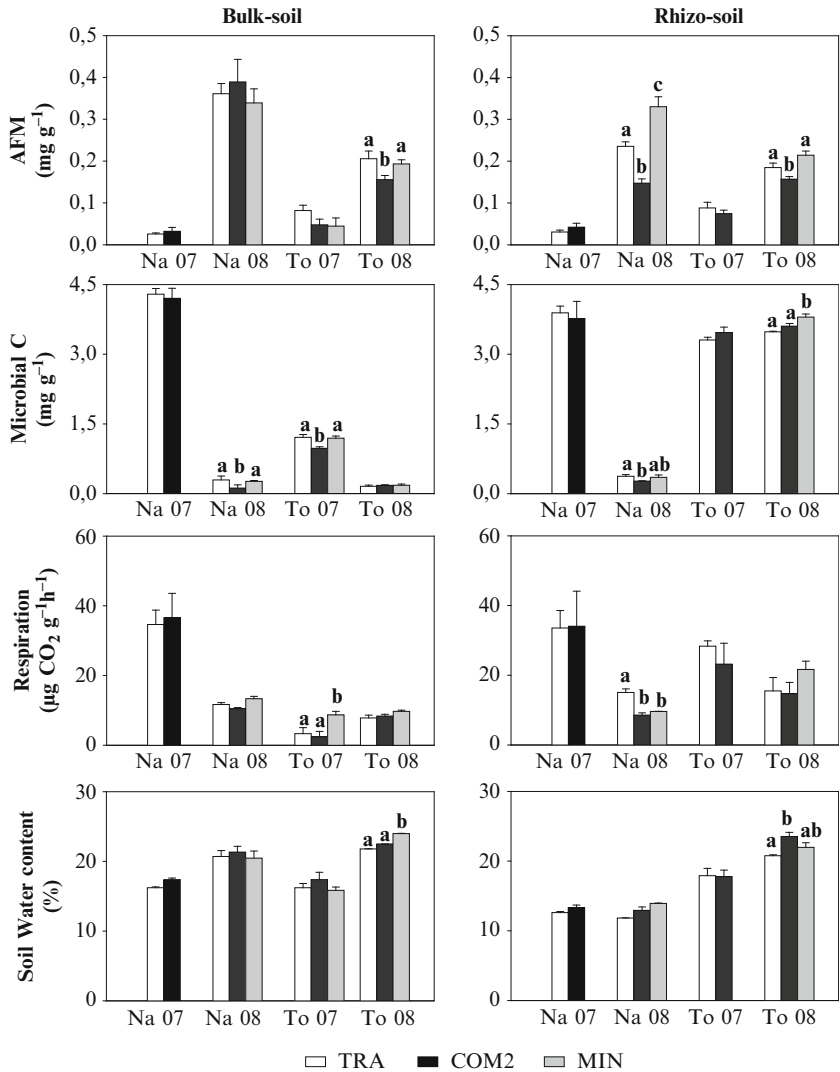


Fig. 6.4 Active fungal mycelium, microbial C, microbial respiration, and water content (mean $\pm$ SE) of soil sampled from Napoli (Na) and Torino (To) experimental sites. *TRA* conventional tillage, *COM-2* compost amendment, *MIN* minimum tillage. *Left-hand-side* figures report values for bulk-soil; *Right-hand-side* figures report values for rhizo-soil. Different letters indicate significant differences between treatments (ANOVA–Holm–Sidak test; $p < 0.05$) within site and year

in the amount of active fungal mycelium was detected in 2008, as compared to TRA and MIN (Fig. 6.4). Moreover, in both 2007 and 2008, COM-2 reduced microbial biomass of bulk soil and rhizo-soil in Napoli, though differences were statistically significant only in 2008 (Fig. 6.4). In Torino, the microbial biomass of COM-2 bulk soil in 2007 was significantly lower than in both TRA and MIN, while in 2008 the rhizo-soil microbial biomass of COM-2 was significantly lower than in MIN (Fig. 6.4). For both Napoli and Torino, the bulk-soil microbial biomass was significantly larger in 2007 than in 2008, though the difference for rhizo-soil microbial biomass between the 2 years was significant only for Napoli (Fig. 6.4).

Soil respiration was significantly affected by *soil* (bulk/rhizo) or *site* as well as by *soil-site* interaction (Table 6.1). A soil respiration significantly lower than MIN and TRA was found for COM-2 in Torino bulk soil in 2007 and in Napoli rhizo soil in 2008, respectively (Fig. 6.4). In 2008, the coefficient of endogenous mineralization (CEM) was significantly affected by both *treatment* and *site* (Fig. 6.5), and by

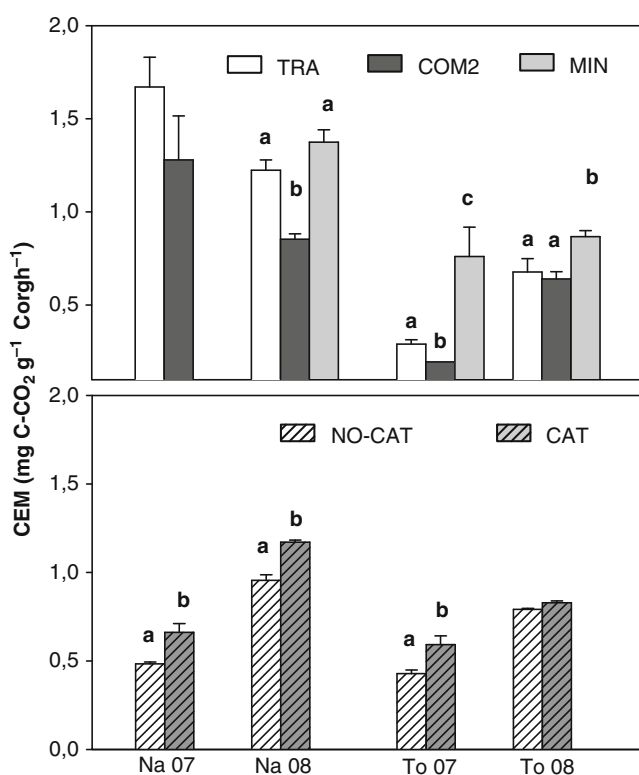


Fig. 6.5 Coefficient of endogenous mineralization (CEM) of soils sampled from the Napoli (Na) and Torino (To) experimental sites. The values are mean $\pm$ SE. TRA conventional tillage, COM-2 compost amendment, MIN minimum tillage. CAT: conventional tillage with addition of biomimetic catalyst, No-CAT: conventional tillage without catalyst. Different letters indicate significant differences among treatments (ANOVA–Holm–Sidak test; $p < 0.05$) within site and year

their interaction. CEM was always lower in COM-2 than in TRA and MIN for both Napoli and Torino field sites.

The general reduction of AFM, microbial biomass and respiration, for both bulk soil and rhizo soil subjected to COM-2 may be explained by the protection that the humified mature compost exerts on bio labile components of soil. This makes them less bio accessible and thus more resistant to microbial degradation (Spaccini et al. 2002; Piccolo et al. 2004).

Another effect of COM-2 was the increase in soil moisture, compared to TRA and MIN. According to Carter (2007), compost amendment improves soil porosity and consequently favors an increase in soil moisture. For both Napoli and Torino sites, water content in bulk soil for the 2008 experimental year was generally larger than for 2007 (Fig. 6.4), whereas the rhizo soil at Napoli had lower water content than the rhizo soil at Torino. More specifically, soil water content tended to be the highest in compost-amended plots at both sites and in both years.

In addition, no difference was found in microbial biomass in soils subjected to TRA and MIN, with the exception of Torino rhizo soil. This result may indicate that the 3-year treatment period is too short to produce a significant improvement in soil biological quality. In fact, various studies (Joergensen and Castillo 2001; Balota et al. 2003; Franchini et al. 2005; Wright et al. 2008; Helgason et al. 2009) indicate that the effects of different agricultural management on soil microbial communities become evident after longer periods (at least 10 years). Nevertheless, AFM and microbial biomass of rhizo soil in MIN were larger in 2008 than in TRA for the Napoli and Torino sites, respectively.

6.7 Effects of the Biomimetic Catalyst

Humic substances comprise the major part of stable organic matter in environmental compartments and their formation and decomposition processes regulate global carbon cycling. An increase in the conformational stability of humus may be achieved by increasing the intermolecular covalent bonds among heterogeneous humic molecules through a photo-oxidative coupling mediated by a biomimetic (enzyme-like) catalyst, such as synthetic water-soluble metal–porphyrins (Piccolo et al. 2005). It was found that soil amendments with the biomimetic catalyst affected the molecular structure of SOM and decreased its biotic degradation, thereby significantly decreasing CO₂ emission from soil (Gelsomino et al. 2010; Piccolo et al. 2011). However, since new molecules added to soil, though apparently harmless and eco-compatible, may deeply alter the behavior of microbial populations through complex and unexpected interaction (biotic and/or abiotic), we studied the impact of the biomimetic catalyst on the dynamics of microbial soil populations in the different experimental fields of the MESCOAGR project.

6.7.1 Microbial Counts

The CAT treatment showed hardly any long-term effects on cultivable microbial populations, as evaluated in both bulk soil and rhizo soil. In fact, no significant difference was found between CAT plots and their control (No-CAT) in any of the experimental sites for the first 2 years of treatment (Figs. 6.6a, b and 6.7a, b).

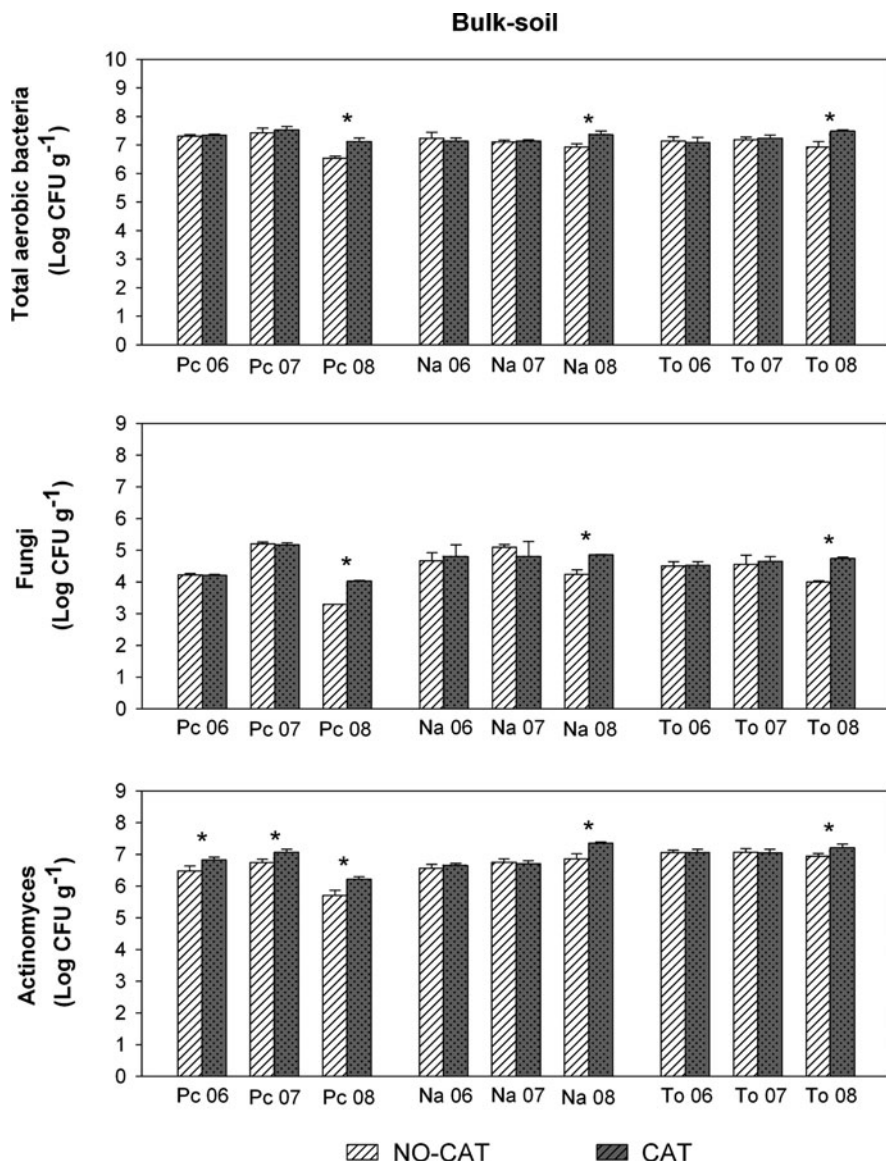


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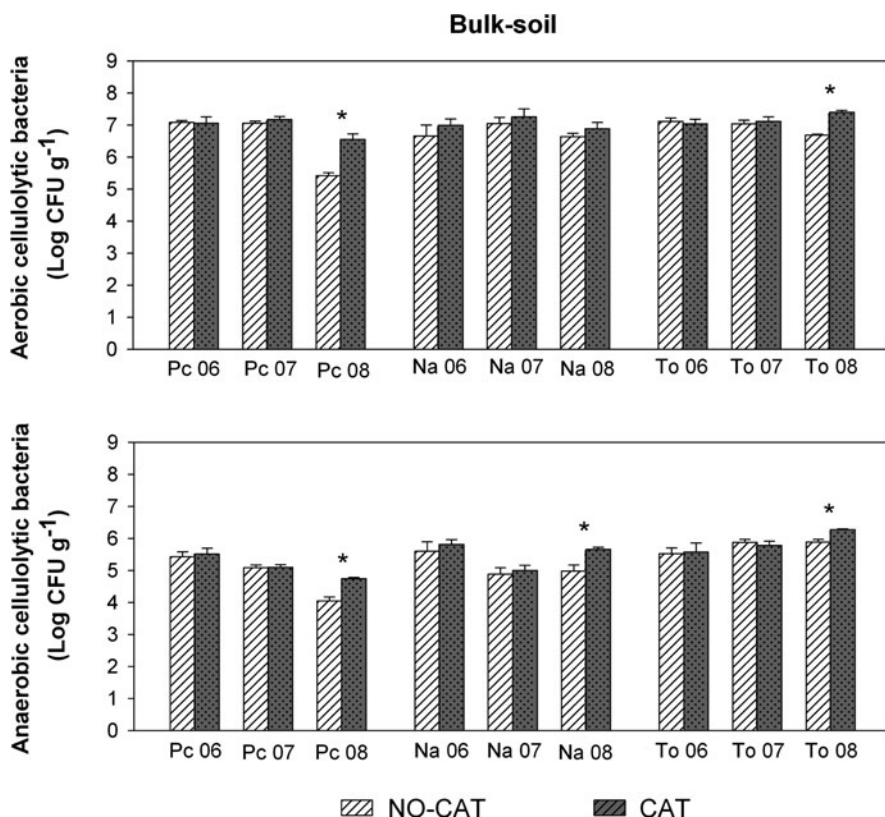


Fig. 6.6 Effect of synthetic metal-porphyrins addition on (a) total aerobic bacteria, fungi, and actinomycetes (mean of Log CFU g⁻¹ of soil $\pm$ SE) in bulk-soils of Piacenza (Pc), Napoli (Na), and Torino (To). Asterisk indicates significant at $p < 0.05$ within site and years. NO-CAT: control, CAT: soil treated with biomimetic catalyst (b) aerobic and anaerobic cellulolytic bacteria (mean of Log CFU g⁻¹ of soil $\pm$ SE) in bulk-soils of Piacenza (Pc), Napoli (Na), and Torino (To). Asterisk indicates significant at $p < 0.05$ within site and years. NO-CAT: control, CAT: biomimetic catalyst

The only exception was in the Piacenza bulk soil that showed an increase in actinomycete populations during the whole experimental period (Fig. 6.6a). This long-term effect was probably due to the firm adsorption of the added metal-porphyrin on the large amount of clay particles present in this soil, thereby resulting in greater catalytic activity upon soil biotic and/or abiotic components.

In the last experimental year (2008), the CAT effect on the bulk-soil was the same in the three different field sites, since it significantly affected the microbial groups directly involved in OM mineralization. In fact, the number of total heterotrophic aerobic bacteria, fungi, actinomycetes, aerobic, and anaerobic cellulolytic bacteria was significantly larger in CAT than in No-CAT by an extent of about 1 Log CFU g⁻¹ cycle (Fig. 6.6a, b).

Conversely, it appears that CAT negatively affected the OM mineralization communities in rhizo soils, although the effect on maize rhizo soil (Piacenza) was different from that on wheat rhizo soil (Napoli and Torino). In particular, CAT did not influence the cellulolytic bacteria in the wheat rhizo soils of both Napoli and Torino with respect to No-CAT for all experimental years (Fig. 6.7a, b). By contrast, CAT significantly affected microbial communities in maize rhizo soil.

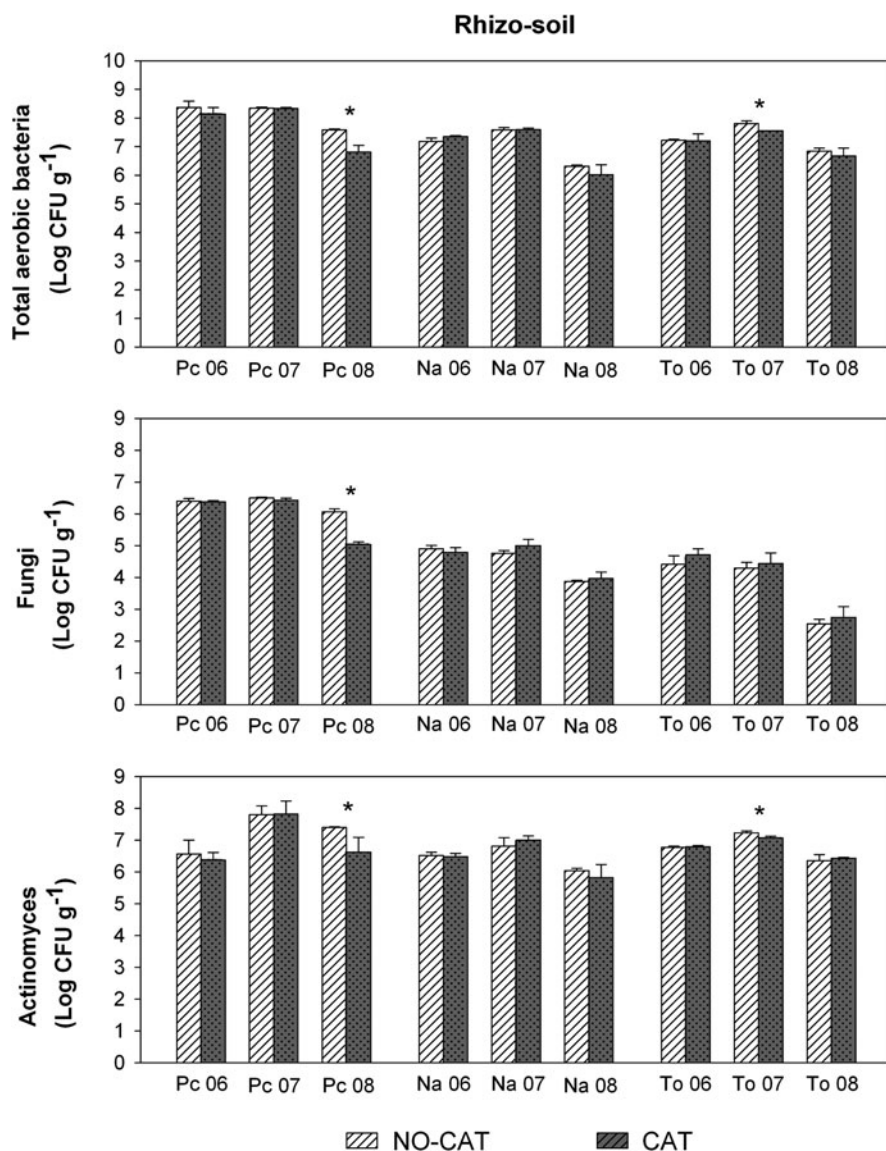


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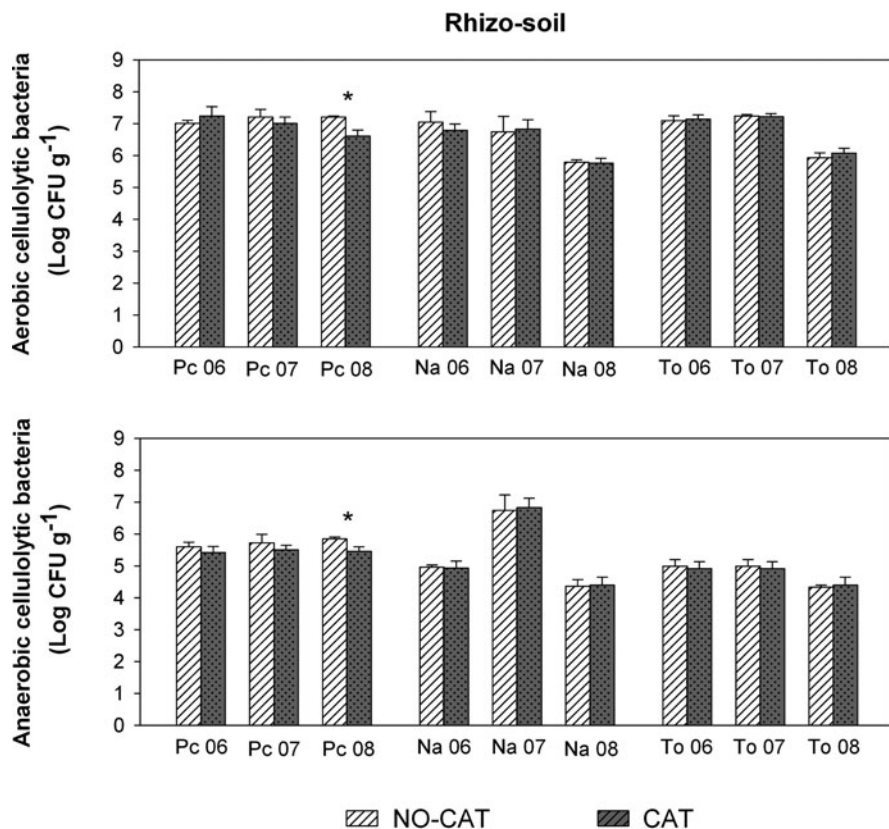


Fig. 6.7 Effect of synthetic metal-porphyrins addition on (a) total aerobic bacteria, fungi, and actinomycetes (mean of Log CFU g⁻¹ of soil $\pm$ SE) in maize rhizosphere of Piacenza agronomic station (Pc), and in wheat rhizosphere of Napoli (Na) and Torino (To) agronomic stations. Asterisk indicates significant at $p < 0.05$ within site and years. NO-CAT: control, CAT: biomimetic catalyst (b) aerobic and anaerobic cellulolytic bacteria (mean of Log CFU g⁻¹ of soil $\pm$ SE) in maize rhizosphere of Piacenza agronomic station (Pc), and in wheat rhizosphere of Napoli (Na) and Torino (To) agronomic stations. Asterisk indicates significant at $p < 0.05$ within site and years. NO-CAT: control, CAT: biomimetic catalyst

In fact, a decrease in the number of all cultivable microorganisms in Piacenza was found after three experimentation years (Fig. 6.7a, b). However, this effect was more extensive in fungi populations which decreased from 6.06 ± 0.09 for NO-CAT to 5.03 ± 0.08 Log CFU g⁻¹ for CAT (Fig. 6.7a).

The different effect of CAT on microbial communities in rhizospheres of maize and wheat may be due to different root systems and root activities. Maize plants have more expanded root systems than wheat, and, thus, explore a greater volume of soil and possibly induce a larger root exudation in the rhizosphere, to promote microbial growth. It is known that when root exudates serve as sole source of C and energy for soil microbes, root exudation is 2–2.6 times greater than in the case of

aseptically grown plants (Vancura et al. 1977; Prikryl and Vancura 1980). Thus, the lower content of soil microbes in CAT suggests that a reduction in root exudate stimulation has occurred, possibly because another source of C and energy was made available to microbes by the CAT treatment. In fact, the enhanced growth of microbial cells following root exudate stimulation is attributed to carbohydrates in exudates (van Overbeek and van Elsas 1995). Therefore in the rhizosphere, the activity of the catalyst on SOM may lead to release of novel organic molecules. The consumption of such molecules by microbes may have depressed the increase of the root exudates/microbial growth cycle observed in No-CAT rhizo soils. Gelsomino et al. (2010) added the biomimetic catalyst on a microcosm soil with and without maize plants and measured CO₂ respiration (see also Chap. 10). They found that while respiration was reduced in catalyst-treated bare soils, there was an enhanced respiration when maize plants were present. Although these results are intriguing, they are difficult to compare since our findings refer to maize rhizo-soil microbial communities and were obtained from field experiments rather than in microcosms.

Nevertheless, the significant decrease observed for maize rhizo soil under CAT treatment suggests that maize plants appear as more suitable indicators than wheat plants in highlighting the effect of the biomimetic catalyst. Furthermore, our findings indicate that the biomimetic catalysts added to soil did not appear to be harmful to cultivable microbial communities, since no lethal effect was recorded.

6.7.2 AFM, Microbial Biomass and Activity

CAT significantly affected soil respiration and CEM (Table 6.2). Moreover, significant differences between bulk soil and rhizo soil were found for all microbial parameters (Table 6.2). The differences between sites were significant for C_{mic} in 2007 and 2008, as well as for respiration and CEM in 2008. Moreover, a significant interaction *soil* $\times$ *site* was observed for AFM and C_{mic} (Table 6.2).

As for bulk soils, AFM in Napoli was larger in CAT than in No-CAT, though the differences were not significant (Fig. 6.8), while in Torino AFM was first significantly lower in CAT than in No-CAT in 2007 and, then, significantly larger in 2008 (Fig. 6.8). In the case of rhizo soils AFM found in CAT treatments was always lower than in No-CAT, but the difference was significant only for Torino in 2007 (Fig. 6.8).

Microbial biomass was not found significantly different between CAT and No-CAT in either bulk or rhizo soils throughout the experimental period for either Napoli or Torino (Fig. 6.8).

Respiration showed a similar increasing trend from No-CAT to CAT treatments in both Napoli and Torino and for either bulk soil or rhizo soil. However, the increase was significant only for Napoli bulk soil in 2007 and Napoli rhizo soil in 2008 (Fig. 6.8).

Table 6.2 Levels of significance (p values from ANOVA) for effects of a biomimetic catalyst (iron-porphyrin) addition to soil on microbial biomass and activity in bulk soil and rhizo soil at Napoli and Torino sites, and differences between years

	AFM		C_{mic}		Respiration	CEM	
	dF	p				dF	p
<i>2007 (Three-way)</i>						<i>2007 (Two-way)</i>	
Treatments (CAT/NO-CAT)	1	0.073	0.510	0.040		Treatments (CAT/NO-CAT)	1 0.010
Soil (Bulk–Rhizo)	1	0.019	0.004	0.005		Site (Napoli–Torino)	1 0.165
Site (Napoli–Torino)	1	0.422	0.015	0.893		Treatments $\times$ site	1 0.850
Treatments $\times$ soil	1	0.166	0.125	0.138			
Treatments $\times$ site	1	0.159	0.857	0.710			
Soil $\times$ site	1	0.931	< 0.001	0.365			
Treatments $\times$ soil $\times$ site	1	0.571	0.133	0.522			
<i>2008 (Three-way)</i>						<i>2008 (Two-way)</i>	
Treatments (CAT/NO-CAT)	1	0.275	0.874	0.016		Treatments (CAT/NO-CAT)	1 0.002
Soil (Bulk–Rhizo)	1	< 0.001	< 0.001	< 0.001		Site (Napoli–Torino)	1 < 0.001
Site (Napoli–Torino)	1	0.154	0.003	< 0.001		Treatments $\times$ Site	1 0.008
Treatments $\times$ soil	1	0.140	0.668	0.854			
Treatments $\times$ site	1	0.816	0.487	0.049			
Soil $\times$ site	1	0.002	< 0.001	0.988			
Treatments $\times$ soil $\times$ site	1	0.864	0.575	0.575			
<i>2007–2008 (Three-way)</i>						<i>2007–2008 (Two-way)</i>	
Years (2007–2008)	1	< 0.001	< 0.001	< 0.001		Years (2007–2008)	1 < 0.001
Site (Napoli–Torino)	1	0.280	0.030	0.033		Site (Napoli–Torino)	1 0.008
Soil (Bulk–Rhizo)	1	< 0.001	< 0.001	< 0.001		Years $\times$ site	1 0.082
Years $\times$ site	1	0.099	< 0.001	0.050			
Years $\times$ soil	1	< 0.001	0.003	0.680			
Site $\times$ soil	1	< 0.001	0.025	0.505			
Years $\times$ site $\times$ soil	1	< 0.001	< 0.001	0.512			

dF degree of freedom, AFM active fungal mycelium, C_{mic} microbial carbon, CEM coefficient of endogenous mineralization, CAT conventional tillage with addition of biomimetic catalyst, $NO-CAT$ conventional tillage without catalyst. Values in bold are statistically significant

The coefficient of endogenous mineralization (CEM) in Napoli was significantly larger in CAT than in No-CAT for both 2007 and 2008 years, while this was true for Torino only in 2007 (Fig. 6.5). Regardless of treatment, CEM for Napoli soils was greater than for Torino (Fig. 6.5).

When comparing wheat soil and maize soil (No-CAT/TRA), C_{mic} and respiration showed significantly lower values in wheat soil ($p = 0.045$ and $p = 0.029$, respectively). Water content in bulk soils was very similar for Napoli and Torino in both years (Fig. 6.8). Rhizo-soil water content was lower in Napoli than in Torino.

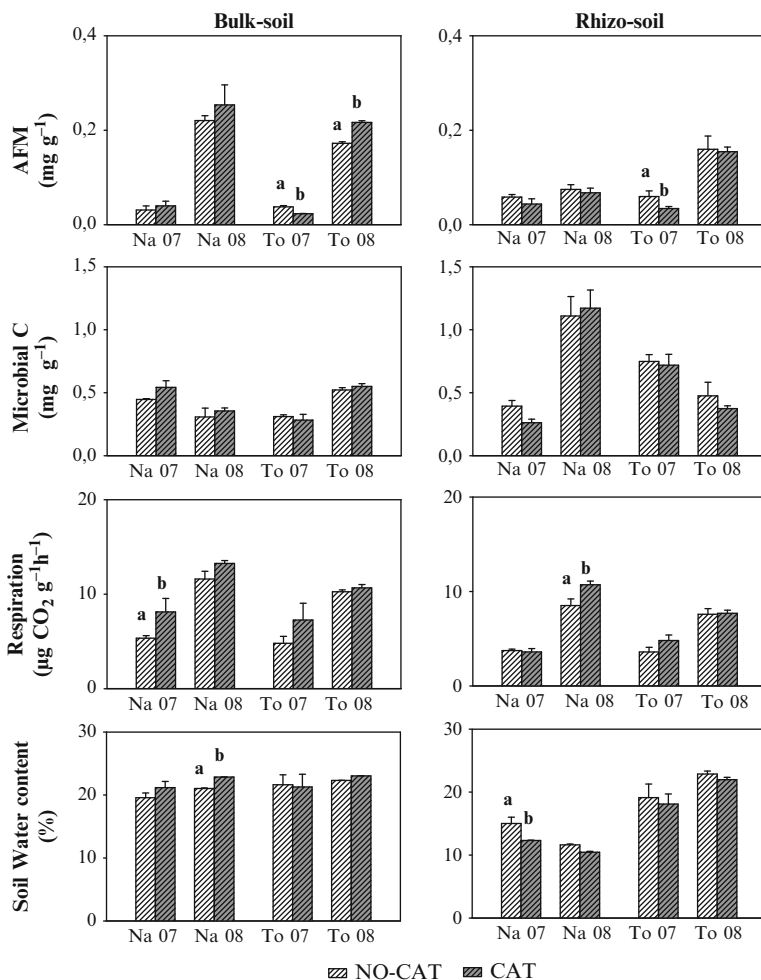


Fig. 6.8 Active fungal mycelium, microbial C, microbial respiration and water content (mean $\pm$ SE) of soil sampled from Napoli (Na) and Torino (To) experimental sites. CAT: biomimetic catalyst, NO-CAT: control. Figures on the *left* report values for bulk-soil; figures on the *right* report values for rhizo soil. Different letters indicate significant differences between treatments (ANOVA–Holm–Sidak test; $p < 0.05$) within site and years

CAT addition increased water content in bulk soil with respect to No-CAT, whereas it had an opposite effect in rhizo soil (Fig. 6.8).

CAT increased AFM, and microbial biomass in bulk soils, but had an opposite effect in rhizo soil, well in agreement with plate-count results for total aerobic bacteria, cellulolytic bacteria, fungi, and actinomycetes. CAT increased respiration in both bulk and rhizo soils, thus suggesting, in line with CEM values, that the in situ photo-polymerization of SOM unexpectedly favors instead of limiting CO₂

emissions. This result is consistent with the larger CO₂ fluxes measured in the field from CAT soils compared to No-CAT, although the emissions include root respiration (see Chap. 9). Moreover, our findings are in line with the cited microcosm experiment (Gelsomino et al. 2010) that revealed that the addition of iron–porphyrin significantly reduced CO₂ efflux from the unplanted soil, whereas CO₂ emission was stimulated when maize plants were present. Gelsomino et al. (2010) hypothesized that the coarser root system induced by iron–porphyrin favored enhanced destruction of soil macroaggregates, thus exposing physically protected SOM to microbial decomposition. However, they were not able to quantify the contribution to CO₂ emission from soil of autotrophic respiration (maize roots) and heterotrophic respiration (rhizosphere microorganisms).

Our data refer to the effect of CAT treatment on soils under wheat and they do not take into account root respiration. Moreover, we found that respiration increased in both the rhizo and bulk soils. Therefore, at least for bulk soils, the explanation proposed by Gelsomino et al. (2010) should be definitely excluded. However, there were contrasting responses of microbial communities to CAT for either bulk or rhizo soils, and it is likely that root systems inhibit growth of the microbial community. Despite the observed evidence of CO₂ being released as much or more in CAT than in No-CAT, the catalyst-assisted *in situ* photo-polymerization of SOM has been shown to sequester organic C throughout the experimentation period in all sites (see Chap. 4).

These contrasting results cannot be yet totally explained since the mechanism underlying the interactions among the catalyst, microbial community, and root systems is complex. However, a possible reason for such an opposite behavior may be the fact that substrates for oxidative photo-polymerization are the phenolic or oxidized aromatic moieties of SOM, which produce the free radicals, whose coupling increases covalent bonds among humic molecules. These aromatic photo-polymerized components of SOM certainly become more biologically stable in soil, thus possibly explaining the reduction of AFM in some cases. Consequently, the carbon-chain alkyl compounds of SOM may result more easily accessible to microbial degradation due to alteration of humic conformations following separation of the photo-polymerized aromatic moieties.

It is also interesting to note that water content in rhizo soils is lower in CAT than in No-CAT, while the opposite is true for bulk soils. This may be due to the fact that the interaction of root systems with the catalyst induces an alteration of the surrounding soil structure, thus limiting the water retention capacity. Such alteration may also influence the size of the microbial community.

When comparing results for wheat and maize soils, it is evident that microbial biomass and activity are larger under maize. Given that wheat and maize grow in different seasons, climatic conditions could at least in part explain such differences (Mahmood et al. 2005). However, it is important to recall that different plant species produce different rhizosphere effects (Vancura et al. 1977; Cheng et al. 2003). There was a weak rhizosphere effect on fungal communities at both sites under either maize or wheat. In contrast a positive rhizosphere effect on C_{mic} was observed under maize at Torino, where, an increase of microbial biomass was accompanied by an increase in respiration.

6.8 Conclusion and Future Recommendations

First of all, our results highlight the importance of combining different approaches to obtain complementary information on the microbiological status of agricultural soils.

Amendment with compost appears to have a promising environmental application, although its use depends on soil texture and clay content, as shown by our studied sites. In fact, compost was found to decrease cultivable microorganisms, microbial carbon, and coefficient of SOM mineralization in clayey soils, possibly due to an increased physical and chemical protection of organic matter from microbial attack. On the other hand, such an effect was not equally evident in soil with lower clay content. Van Elsas et al. (2007) denied direct correlation between abundance of microbial populations and their activities (e.g., N-fixation and cellulolytic activities). The activities are sometimes enhanced by an improved nutrient availability caused by lower competition among microbial cells and by a large concentration of “microbivores” (microbial-feeding microfauna such as mites and nematodes), which keep bacterial abundance at a minimum. Thus, a poliphasic approach including microfauna analyses is necessary to fully understand the complex interactions within the soil food web.

The use of the biomimetic catalyst to fix and/or stabilize soil carbon by photopolymerization caused contrasting responses of soil microbial community. It became evident from concomitant results of other MESCOSAGR groups that the different effects of the catalyst depend on whether the soil is either planted or bare, but also on plant species (maize or wheat), regardless of soil texture and climatic conditions. Such results are consistent with the results obtained by the biotechnological group of MESCOSAGR project (see Chap. 8).

It is thus hoped that further investigations will be conducted, to include analysis of microfauna–microflora interactions, in order to reach a deeper understanding of the long-term effects of compost and metal–porphyrin catalyst on carbon sequestration in soils cultivated with different plant species.

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Chapter 7

Effects of Methods of Carbon Sequestration in Soil on Biochemical Indicators of Soil Quality

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Abstract Here we describe the effects of carbon sequestration managements on soil enzymatic activities and PLFA patterns, as viable parameters to establish soil biochemical quality and its changes. We extensively review the available scientific literature related to experimental results on soil enzymatic activities and PLFA values from different soil treatments. This knowledge was then compared with the experimental results obtained within the MESCOSAGR project. It was found that MESCOSAGR findings are well in agreement with literature, and they show that the use of mature compost or adoption of reduced tillage practices provides an improvement of soil quality, as shown by a general increase in different enzymatic activities. The carbon sequestration method based on the in situ photo-polymerization of soil organic matter catalyzed by a water-soluble iron–porphyrin spread on soil did not show significant difference in soil biochemical quality from control. Changes in microbial communities at taxonomical level have also been identified with PLFA determinations, but these changes were usually site-specific, and mostly related to expression of ecological functions. Our work confirms the importance of linking structural with functional measurements when assessing the response of soil microbial communities to any experimental factor.

7.1 Introduction

Soil is a natural resource that is not renewable at the human time scale and often subjected to a range of alteration events due to human or natural activities. One of the most striking features of soil is its biological complexity, still largely unknown. Soil is indeed the most biological diverse environment on Earth (Dance 2008), and

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its main ecological and productive functions are driven and sustained by the presence and activity of microorganisms (Young and Crawford 2004).

Scientists are still largely debating about the estimations of the size of microbial communities in soils. One of the first estimations, published in 1990, pointed to about 4,000 different bacterial genomes per gram of soil (Torsvik et al. 1990). Later studies have first moved the estimated number of species per gram of soil to 830,000 (Gans et al. 2005) and, then, back down to a number between 20,000 and 50,000 species (Roesch et al. 2007). Of course these estimations are affected by a numbers of factors, starting from the type of soil considered and the methods used for the estimation. Amann et al. (1995) reported that more than 99% of the bacterial species in soils are unculturable, and this has been widely confirmed by recent studies (Handelsman 2004; Deutschbauer et al. 2006). Research is now mining at molecular level into this widely unknown unculturable majority, by taking advantage of new technologies which allow a fine-scale resolution of DNA, RNA and proteins in soils. These approaches will most probably give relevant outcomes at both basic and applied science level in the next years, but at the moment the level of complexity is so high that is difficult to translate the amount of information obtained in, for example, indexes of soil quality and/or alterations due to soil treatments.

Soil scientists have been studying the structure and most importantly the functionality of soil microbial communities for decades, by developing and optimizing methods that are now still applied and widely accepted, even at regulatory levels. We have focused on two of these methods: enzymatic activities and phospholipids fatty acid (PLFA) analyses. Methods and literature evidence are firstly discussed in relation to soil management practices aimed at carbon sequestration, namely amendment with compost and other organic residues, and reduced or no tillage managements. In the second part of the chapter, evidence from MESCOSAGR project is discussed in relation to literature review. Original outcomes about possible effects of the third C sequestration strategy considered in MESCOSAGR (organic matter photo-polymerization under a biomimetic catalyst) on soil PLFAs and enzymes are also presented.

7.2 Enzymatic Activities and Soil Carbon Sequestration Strategies

The expression “enzymatic activities” is usually preferred to “enzymes,” the reason being that applied methods do not isolate and measure the enzyme itself in soils, but quantify (mainly by absorbance or fluorescence) the rate of transformation of an enzyme substrate added to soil. Already in the 1940s a paper was published reporting the effect of copper nitrogen complex on soil potential nitrification, as assessed by this substrate-induced approach (Lees 1946), and it was followed by a series of pioneering works on the location and activities of enzymes in soils

Table 7.1 Number of published literature on enzymes and PLFAs in soil as related to compost and tillage treatments

Search word	<2008	2008	2009	2010	2011	Total
Soil* AND enzym*	10,388	1,188	1,290	1,337	135	14,338
Soil* AND enzym* AND compost	220	48	55	56	3	382
Soil* AND enzym* AND tillage	124	16	27	33	3	203
Soil* AND PLFA*	502	113	95	109	18	819
Soil* AND PLFA* AND compost	19	4	1	5	0	29
Soil* AND PLFA* AND tillage	20	4	3	6	3	36

The enquiry was carried out on Scopus database in February 2011 among titles, abstracts and keywords

(McLaren 1954; McLaren et al. 1957). From then on, the assessment of enzymatic activities (or more generally biological activities, as in the case of nitrification, where a series of enzymatic activities is involved) has become widely popular. A search on the Scopus scientific database using “soil*” and “enzym*” as search words among titles, abstracts and keywords gives a total 14,332 published papers, with more than a thousand papers per year (Table 7.1). If the search is restricted adding either “compost” or “tillage,” the number of published papers is, respectively, 382 and 203, with a slight increase in the last years.

Numerous reviews about enzymatic activities have been published in the last years (e.g., Dick 1992; Tabatabai et al. 2002; Nannipieri et al. 2003; Caldwell 2005), as well as a number of books (Burns 1978; Dick and Burns 2002). Thus, the aim of this chapter is not to provide a further review on the topic but to analyse the evidence available in literature on the effects of organic fertilization and tillage on soil enzymatic activities and to assess whether some general conclusions can be drawn from this critical bibliographic investigation.

Soil enzymatic activities are related to the majority of ecological processes in soils such as soil organic matter decomposition, cycling of nutrients, and detoxification of undesired compounds, such as pesticides and other organic contaminants. Enzymes play a main role in relation to presence and activities of soil microorganisms, since their catalytic activity toward transformation of organic substrates allows liberation of the necessary energy for their activities (Kiss et al. 1978) and promotes soil fertility by releasing nutrients for plants growth. Soil enzyme activities have been suggested as suitable indicators of soil quality for a number of reasons: (1) they are an index of soil microbial activity and, thus, they are strictly related to nutrient cycles and transformations; (2) they may rapidly respond to changes in soil caused by both natural and anthropogenic factors; (3) they are easy to measure (Calderon et al. 2000; Drijber et al. 2000; Nannipieri et al. 2002). The information given by a single enzymatic activity is of course important but limited. This is why most works usually consider a range of enzymatic activities, eventually condensing all information in numerical indices obtained by different approaches. In a recent review paper, 13 indexes based on soil enzymatic activities were discussed (Bastida et al. 2008).

Here we present the outcomes of an extended bibliographic review carried out in order to assess, identify, and interpret possible trends in the response of the main experimental factors studied in MESCOSAGR project: organic fertilization (Table 7.2) and reduced or no tillage (Table 7.3). Among the papers cited in Table 7.1, only the ones which allowed extrapolation of quantitative data were selected.

Fourteen studies dealing with the effects of organic fertilizers on soil enzymatic activities have been reviewed. Twelve enzymatic activities have been considered, namely arylsulphatase, β -glucosidase, phosphatase, fluorescein diacetate hydrolysis activity (FDA), urease, dehydrogenase, invertase, phenoloxidase, catalase, protease, nitrate reductase and amylase. For each study the type and amount of organic fertilizer applied is reported, together with information (when available) on soil texture and taxonomy. Soil textures ranged from sandy to clay, whereas 11 different soil types were considered (Table 7.2).

Arylsulphatase activity is usually assessed by adding a substrate such as *p*-nitrophenylsulphate in soil, and quantifying the amount of *p*-nitrophenol produced in time. Up to our knowledge, it is the only enzyme of the S cycle whose activity is assessed in soil. However, it is considered quite representative of the mineralization of organic S in soils, since sulfate esters represent a large fraction (25–93%) of the soil total S (Elsgaard et al. 2002). Six papers were considered about the effects of compost amendment on arylsulphatase (Table 7.2). Five papers indicated an increase in arylsulphatase activity as a result of organic additions, whereas only one (Abdelbasset et al. 2011) indicated no relevant effects after application of up to 80 ton ha⁻¹ of municipal solid waste (MSW) compost to a clayey-loamy soil cropped with *Triticum durum* (although the use of sewage sludge had instead a positive effect). In two of the works, arylsulphate activity was more than doubled as a result of application of 2–4 ton ha⁻¹ of MSW compost (Albiach et al. 2000) or of composted red clover corresponding to 416 kg of total N ha⁻¹ (Elfstrand et al. 2007a). Two other reports dealt with a maize field amended for several years with MSW compost and sewage sludge (Puglisi et al. 2006) and with the application of compost rates up to 45 ton ha⁻¹ in a greenhouse and in an open field under Mediterranean conditions (Iovieno et al. 2009). Finally, Darby et al. (2006) assessed the effects of compost from dairy manure solids (56 ton ha⁻¹) on sweet corn plots in Oregon.

β -Glucosidase is one of the enzymatic activities involved in C cycling in soils. It is usually assessed using *p*-nitrophenyl-b-D-galactoside as a substrate, and it thus gives an indication of the activity of enzymes involved in cellulose degradation, specifically in the hydrolysis of β -1,4 bonds in β -glucopiranosides. Eight papers were considered here (Table 7.2). Five of them dealt with MSW compost, one with municipal food waste (MFW) compost, one with compost from manure mixed with leguminous residues, and another one with an unspecified compost. Concentrations considered ranged from 5 to 80 ton ha⁻¹. According to five studies, β -glucosidase activity was increased after amendment with compost from MSW (Garcia-Gil et al. 2000; Crecchio et al. 2004; Hojati and Nourbakhsh 2009; Abdelbasset et al. 2011), MFW (Iovieno et al. 2009) and manure mixed with leguminous residues (Laudicina

Table 7.2 Analysis of literature info on the effects of organic fertilization on soil enzymatic activities

Enzyme activity	Organic fertilizer applied	Effect	Soil texture	Soil taxonomy
S cycle				
Arylsulphatase	MSW compost (24 ton ha ⁻¹ year ⁻¹)	↑↑ Albiach et al. (2000)	Sandy-silty loam	Xerorthent
	Composted dairy manure (56 ton ha ⁻¹ year ⁻¹)	↑ Darby et al. (2006)	Silty loam	Chealis
	MSW compost (25 ton ha ⁻¹ year ⁻¹)	↑ Puglisi et al. (2006)	Sandy loam	Alluvial hydromorphic
	MFW compost (45 ton ha ⁻¹ in 3 rates)	↑ Iovieno et al. (2009)	–	Calcaric Cambisol
	Composted red clover (15 ton ha ⁻¹)	↑↑ Elfstrand et al. (2007a)	Silty clay loam	–
	MSW compost (80 ton ha ⁻¹)	- Abdelbasset et al. (2011)	Clay loam	Mollisol
C cycle				
β-Glucosidase	MSW compost (80 ton ha ⁻¹)	↑ Garcia-Gil et al. (2000)	Sandy	Typic Haploxeralf
	MSW compost (24 ton ha ⁻¹ year ⁻¹)	↑ Crecchio et al. (2004)	Clay	Typic Chromoxerert
	MSW compost (25 ton ha ⁻¹ year ⁻¹)	↓ Puglisi et al. (2006)	Sandy loam	Alluvial hydromorphic
	Compost (5 ton ha ⁻¹ year ⁻¹)	↓ Nayak et al. (2007)	Sandy clay loam	Aeric Endoaquept
	MSW compost (100 ton ha ⁻¹)	↑ Hojati and Nourbakhsh (2009)	Silty clay loam	Typic Haplargid
	MFW compost (45 ton ha ⁻¹ in 3 rates)	- Iovieno et al. (2009)	–	Calcaric Cambisol
	Compost from manure (30 ton ha ⁻¹ year ⁻¹)	↑ Laudicina et al. (2010)	–	Hortic Cambisol
	MSW compost (80 ton ha ⁻¹)	↑ Abdelbasset et al. (2011)	Clay loam	Mollisol
	MSW compost (25 ton ha ⁻¹ year ⁻¹)	↑ Puglisi et al. (2006)	Sandy loam	Alluvial hydromorphic
Invertase	Compost (5 ton ha ⁻¹ year ⁻¹)	↓ Nayak et al. (2007)	Sandy clay loam	Aeric Endoaquept
	MSW compost (25 ton ha ⁻¹ year ⁻¹)	↑ Puglisi et al. (2006)	Sandy loam	Alluvial hydromorphic
Phenoloxidase	MSW compost (25 ton ha ⁻¹ year ⁻¹)	↑ Puglisi et al. (2006)	Sandy loam	Alluvial hydromorphic
Amylase	MSW compost (15 ton ha ⁻¹)	↑ Pramanik et al. (2010)	Clay	Aqualfs
P cycle				
Phosphatase	MSW compost (24 ton ha ⁻¹ year ⁻¹)	↑↑ Albiach et al. (2000)	Sandy-silty loam	Xerorthent
	MSW compost (80 ton ha ⁻¹)	↓ Garcia-Gil et al. (2000)	Sandy	Typic Haploxeralf
	MSW compost (24 ton ha ⁻¹ year ⁻¹)	↑ Crecchio et al. (2004)	Clay	Typic Chromoxerert
	MFW compost (27 ton ha ⁻¹)	↑↑ Lee et al. (2004)	–	–
	MSW compost (25 ton ha ⁻¹ year ⁻¹)	↑↑ Puglisi et al. (2006)	Sandy loam	Alluvial hydromorphic
	Composted red clover (15 ton ha ⁻¹)	↑↑ Elfstrand et al. (2007a)	Silty clay loam	–

(continued)

Table 7.2 (continued)

Enzyme activity	Organic fertilizer applied	Effect	Soil texture	Soil taxonomy
	MFW compost (45 ton ha ⁻¹ in 3 rates)	- Iovieno et al. (2009)	–	Calcaric Cambisol
	Compost (30 ton ha ⁻¹ year ⁻¹)	↑ Laudicina et al. (2010)	–	Hortic Cambisol
	MSW compost (15 ton ha ⁻¹)	↑ Pramanik et al. (2010)	Clay	Aqualfs
	MSW compost (80 ton ha ⁻¹)	↑ Abdelbasset et al. (2011)	Clay loam	Mollisol
N cycle				
Urease	MSW compost (24 ton ha ⁻¹ year ⁻¹)	↑ Albiach et al. (2000)	Sandy-silty loam	Xerorthent
	MSW compost (80 ton ha ⁻¹)	- Garcia-Gil et al. (2000)	Sandy	Typic Haploxeralf
	MSW compost (24 ton ha ⁻¹ year ⁻¹)	↑ Crecchio et al. (2004)	Clay	Typic Chromoxerert
	MSW compost (6 ton ha ⁻¹)	↑ Bhattacharyya et al. (2005)	–	Typic fluvaquent
	MSW compost (25 ton ha ⁻¹ year ⁻¹)	- Puglisi et al. (2006)	Sandy loam	Alluvial hydromorphic
	Compost (5 ton ha ⁻¹ year ⁻¹)	↑ Nayak et al. (2007)	Sandy clay loam	Aeric Endoaquept
	Compost (30 ton ha ⁻¹ year ⁻¹)	↑↑ Laudicina et al. (2010)	–	Hortic Cambisol
	MSW compost (15 ton ha ⁻¹)	↑ Pramanik et al. (2010)	Clay	Aqualfs
	MSW compost (80 ton ha ⁻¹)	↑ Abdelbasset et al. (2011)	Clay loamy	Mollisol
	MSW compost (80 ton ha ⁻¹)	- Garcia-Gil et al. (2000)	Sandy	Typic Haploxeralf
Protease	MSW compost (24 ton ha ⁻¹ year ⁻¹)	- Crecchio et al. (2004)	Clay	Typic Chromoxerert
	Composted red clover (15 ton ha ⁻¹)	↑↑ Elfstrand et al. (2007a)	Silty-clay-loam	–
	MSW compost (15 ton ha ⁻¹)	↑ Pramanik et al. (2010)	Clay	Aqualfs
	MSW compost (24 ton ha ⁻¹ year ⁻¹)	↑ Crecchio et al. (2004)	Clay	Typic Chromoxerert
Nitrate reductase				
Total microbial activity				
FDA	Composted dairy manure (56 ton ha ⁻¹ year ⁻¹)	↑ Darby et al. (2006)	Silt loam	Chealis
	Compost (5 ton ha ⁻¹ year ⁻¹)	- Nayak et al. (2007)	Sandy clay loam	Aeric Endoaquept
	MFW compost (45 ton ha ⁻¹ in 3 rates)	↑ Iovieno et al. (2009)	–	Calcaric Cambisol
Dehydrogenase	MSW compost (24 ton ha ⁻¹ year ⁻¹)	↑ Albiach et al. (2000)	Sandy-silty loam	Xerorthent
	MSW compost (80 ton ha ⁻¹)	↑↑ Garcia-Gil et al. (2000)	Sandy	Typic Haploxeralf
	MSW compost (24 ton ha ⁻¹ year ⁻¹)	↑ Crecchio et al. (2004)	Clay	Typic Chromoxerert
	MFW compost (27 ton ha ⁻¹)	↑↑ Lee et al. (2004)	–	–

(continued)

Table 7.2 (continued)

Enzyme activity	Organic fertilizer applied	Effect	Soil texture	Soil taxonomy
Catalase	MSW compost (25 ton ha ⁻¹ year ⁻¹)	↑ Puglisi et al. (2006)	Sandy loam	Alluvial hydromorphic
	Compost (5 ton ha ⁻¹ year ⁻¹)	↑ Nayak et al. (2007)	Sandy clay loam	Aeric Endoaquept
	Compost (30 ton ha ⁻¹ year ⁻¹)	↑ Laudicina et al. (2010)	–	Hortic Cambisol
	MSW compost (80 ton ha ⁻¹)	↑ Abdelbasset et al. (2011)	Clay loamy	Mollisol
	MSW compost (80 ton ha ⁻¹)	↑ Garcia-Gil et al. (2000)	Sandy	Typic Haploxeralf
	MSW compost (80 ton ha ⁻¹)	↑ Abdelbasset et al. (2011)	Clay loamy	Mollisol

For each enzymatic activity the referred biogeochemical cycle is reported. (↑, ↓ Increase or decrease of less than 100%; ↑↑, ↓↓ increase or decrease of more than 100%; - no effect)

et al. 2010). Differently from what reported above for arylsulphatase, no differences were found in β -glucosidase activity according to Iovieno et al. (2009). Conversely, Puglisi et al. (2006) and Nayak et al. (2007) even found a slight but significant decrease, possibly due to presence of toxic trace elements in MSW compost and to the low amount applied (5 ton ha⁻¹), respectively.

Another important enzymatic activity involved in soil C cycling is invertase. As for urease, for which urea is the substrate, invertase is the only other hydrolase assessed using its natural substrate, namely sucrose (Speir et al. 2002). Only two papers dealing with effects of compost on invertase activity were found in literature (Table 7.2). Puglisi et al. (2006) found that in a sandy loam soil amended with 25 ton ha⁻¹ of MSW compost, invertase was significantly enhanced, while according to Nayak et al. (2007) invertase activity was instead reduced in a soil of similar texture (sandy clay loam). In the latter, however, only 5 ton ha⁻¹ of compost were tested, and the presence of clays might as well played a role in adsorbing the enzyme and thus reducing its activity (Gianfreda et al. 1991).

Another C cycling enzymatic activity considered here was phenoloxidase, involved in organic matter degradation. Only one study (Puglisi et al. 2006) was found that showed a significant increase in phenoloxidase activity after compost amendment. It was also found that another C cycling enzyme (amylase, responsible for starch degradation) was increased by compost addition at 15 ton ha⁻¹ (Pramanik et al. 2010).

Phosphatases are key enzymes controlling phosphorus turnover and availability for plants. Assayed after addition of the synthetic substrate *p*-nitrophenylphosphate to soil samples, phosphatases control the transformation of organic P to inorganic P through dephosphorylation processes. These enzymes can be assessed under either alkaline or acidic conditions. Alkaline phosphatases are mostly of microbial origin, while acid phosphatases are more of plant or fungal origin, though this is not a strict difference and conditions may differ from soil to soil. Ten scientific papers were considered here (Table 7.2): seven reports analysed acid phosphatase, two of them

discussed alkaline phosphatase (Albiach et al. 2000; Abdelbasset et al. 2011), while only one (Lee et al. 2004) evaluated both forms. Most papers (eight) analysed the effects of MSW compost (rates ranging from 15 to 80 ton ha⁻¹), one paper described the effect of composted red clover (Elfstrand et al. 2007a) and another one reported effects of an unspecified compost (Laudicina et al. 2010). In eight out of ten papers, phosphatase activities were significantly induced by compost addition. A significant reduction was instead found by Garcia-Gil et al. (2000) for a low organic matter sandy soil amended with an MSW compost, though contaminated with significant levels of Zn (1325 mg kg⁻¹), Cu (548 mg kg⁻¹), Ni (81 mg kg⁻¹) and Pb (681 mg kg⁻¹). The authors attributed this phosphatase inhibition to trace elements level and to the large content of soluble P in the amended soil, in agreement with evidence showing an inhibitory effect of inorganic P on phosphatases (Spiers and McGill 1979).

Urease activity is at the basis of nitrogen turnover and soil fertility. Being assessed through determination of the ammonium liberated after soil addition with urea, this enzyme plays a central role in organic nitrogen mineralization, as it is the gateway for nitrification. Urease is sensitive to a number of environmental parameters such as oxygen and trace elements, and it is well correlated with soil quality (Badiane et al. 2001; Coppolecchia et al. 2010). Nine studies were considered here to assess general trends on the effects of organic amendments on urease activity (Table 7.2). Most of these studies have been already cited for the enzymatic activities discussed above. Seven out of nine studies used MSW compost as fertilizer, two of them used an unspecified compost. Amendment rates ranged from 5 to 80 ton ha⁻¹, and, as for other enzymes, a wide range of soil textural types were considered. In most cases, urease activity was significantly increased by compost, and in one case (Laudicina et al. 2010) more than doubled. In one case (Garcia-Gil et al. 2000; Puglisi et al. 2006), no significant effect was found, while in another work (Garcia-Gil et al. 2000) a significant inhibition of urease from MSW compost was even found. These effects did not seem to be related to compost rates, while Nayak et al. (2007) showed significant increase with a rate of only 5 ton ha⁻¹. The inhibition of urease activity can be due to the presence of trace elements as for other enzymes, or, as suggested by Garcia-Gil et al. (2000), to the large content of NH₄⁺ (a urease inhibitor) produced by the activity of proteases present in the MSW compost-amended soil.

Proteases belong to a large family of soil enzymes, and, depending on the substrate used for determination, different protease activities can be assayed. We considered four papers about the effects of compost on soil protease activities (Table 7.2). The used substrates were N α -benzoyl-argininamide (Garcia-Gil et al. 2000; Crecchio et al. 2004), Na-caseinate (Pramanik et al. 2010), and caseine (Elfstrand et al. 2007a). It was found that protease activity was induced by 15 ton ha⁻¹ of composted red clover (Elfstrand et al. 2007a) and 15 ton ha⁻¹ of MSW compost (Pramanik et al. 2010), while according to Crecchio et al. (2004) and Garcia-Gil et al. (2000), the rates of 80 and 24 ton ha⁻¹ of MSW compost did not cause any significant change in protease activities, respectively.

The assessment of nitrate reductase activity in soil can give an important indication of denitrification. Also this activity is enhanced by compost amendment, thus confirming that organic matter addition stimulates microorganism involved in very different cycles (Crecchio et al. 2004).

Fluorescein diacetate (FDA) is a substrate that can be hydrolysed by a variety of nonspecific enzymes and the assessment of FDA hydrolysis activity is thus used as an indicator of total microbial activity in soil (Perucci 1992). Three papers on the effects of composts on FDA in soil were considered here (Table 7.2). It was found that 56 ton ha⁻¹ of composted dairy manure (Darby et al. 2006) and 45 ton ha⁻¹ of MFW compost (Iovieno et al. 2009) significantly increased soil FDA, while a lower dose of 5 ton ha⁻¹ of compost induced no significant changes (Nayak et al. 2007).

Dehydrogenases are ubiquitous in all intact, viable microbial cells: the estimation of dehydrogenase activity as determined by the reduction of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl-tetrazolium chloride (INT) to iodo-nitrophenyl formazan (INTF) is thus widely used as an estimation of soil total microbial activity (Lagomarsino et al. 2009a). In accordance with the results reported above for the other enzymatic activity used to estimate total microbial activity such as FDA, it was found also through the analysis of papers dealing with the dehydrogenase that compost has a general effect of increase of soil microbial activity. Eight papers were considered (Table 7.2), and all of them showed a significant increase (in some cases more than a doubling) of dehydrogenase as the result of the application of doses of compost ranging from 5 to 80 ton ha⁻¹, on different textures and soil types.

Catalase is the third enzymatic indicator of total microbial activity considered here, and it consists of an oxido-reductase associated with aerobic microbial activity (Rodriguez-Kabana and Truelove 1982). Two papers dealing with the effects of compost on soil catalase were found (Garcia-Gil et al. 2000; Abdelbasset et al. 2011), and in line with FDA and dehydrogenase results, they confirmed the stimulation of microbial activity by compost.

Similarly to the effects of organic amendments, also the effects of tillage on soil enzymatic activities were searched in the scientific literature (Table 7.3). Most of the enzymatic activities discussed above in detail have been also considered for tillage effects. Eleven papers in total have been reviewed, covering different soil textural types. Though both no tillage and reduced tillage were included in the bibliographic query, the vast majority of studies considered solely no tillage. Only one (Ramos et al. 2011) assessed the effect of reduced tillage by chisel ploughing on arylsulphatase, β -glucosidase, phosphatase and dehydrogenase activities.

The effect of no tillage in inducing enzymatic activities was even greater and clearer than that observed for amendment of organic materials. Among ten enzymatic activities, nine were generally increased by no tillage: arylsulphatase (5/7), β -glucosidase (6/8), phenoloxidase (1/1), catalase (1/1), phosphatase (8/8), urease (3/4), invertase (1/1), dehydrogenase (6/8) and protease (2/2). In all other cases, no significant difference was reported. In the case of FDA, only one study was found (Nsabimana et al. 2004) that showed no significant differences between control and no till plots for a clay Rhodic Ferrisol.

Table 7.3 Analysis of literature info on the effects of tillage on soil enzymatic activities

Enzyme activity	Tillage	Effect	Soil texture	Soil taxonomy
S cycle				
Arylsulphatase	No tillage	↑ Nsabimana et al. (2004)	Clay	Rhodic Ferisol
	No tillage	- Mijangos et al. (2006)	Clay loam	–
	No tillage (direct drilling)	↑ Melero et al. (2008)	Clay	Chromic Haploxeret
	No tillage	↑ Lagomarsino et al. (2009b)	Silt–clay	Calcaric Gleyic Cambisol
	No tillage	↑ Mikanová et al. (2009)	Clay loam	Orthic Luvisol
	No tillage	↑ Zhang et al. (2010)	–	–
	Reduced tillage (chisel ploughing)	- Ramos et al. (2011)	Clay loam	Hypercalcic Calcisol
C cycle				
β-Glucosidase	No tillage	↑ Mijangos et al. (2006)	Clay loam	–
	No tillage (direct drilling)	↑↑ Melero et al. (2008)	Clay	Chromic Haploxeret
	No tillage	- Mina et al. (2008)	Sandy clay loam	–
	No tillage	↑↑ Lagomarsino et al. (2009b)	Silt–clay	Calcaric Gleyic Cambisol
	No tillage (mouldboard ploughing)	↑ Lopez-Garrido et al. (2010)	–	Eutric Leptosol
	No tillage	↑↑ Ulrich et al. (2010)	Sandy loam	Stagnic Luvisol
	No tillage	- Zhang et al. (2010)	–	–
Phenoloxidase	Reduced tillage (chisel ploughing)	↑ Ramos et al. (2011)	Clay loam	Hypercalcic Calcisol
	No tillage (mouldboard ploughing)	↑ Lopez-Garrido et al. (2010)	–	Eutric Leptosol
Invertase	No tillage	↑ Mikanová et al. (2009)	Clay loam	Orthic Luvisol
P cycle				
Phosphatase	No tillage	↑ Nsabimana et al. (2004)	Clay	Rhodic Ferisol
	No tillage	↑ Mijangos et al. (2006)	Clay loam	–
	No tillage	↑ Roldan et al. (2007)	Clay	Vertisol
	No tillage (direct drilling)	↑ Melero et al. (2008)	Clay	Chromic Haploxeret
	No tillage	↑ Mina et al. (2008)	Sandy clay loam	–
	No tillage	↑↑ Lagomarsino et al. (2009b)	Silt–clay	Calcaric Gleyic Cambisol

(continued)

Table 7.3 (continued)

Enzyme activity	Tillage	Effect	Soil texture	Soil taxonomy
N cycle	No tillage	↑↑ Zhang et al. (2010)	–	–
	Reduced tillage (chisel ploughing)	↑ Ramos et al. (2011)	Clay loam	Hypercalcic Calcisol
	No tillage	– Mina et al. (2008)	Sandy clay loam	–
Urease	No tillage	↑ Mikanová et al. (2009)	Clay loam	Orthic Luvisol
Protease	No tillage	↑ Zhang et al. (2010)	–	–
	No tillage	↑ Qin et al. (2010)	Silt loam	Haplic Cambisol
	No tillage	↑ Mina et al. (2008)	Sandy clay loam	–
	No tillage	↑↑ Zhang et al. (2010)	–	–
Total microbial activity				
FDA	No tillage	– Nsabimana et al. (2004)	Clay	Rhodic Ferisol
Dehydrogenase	No tillage	– Nsabimana et al. (2004)	Clay	Rhodic Ferisol
	No tillage	↑ Mijangos et al. (2006)	Clay loam	–
	No tillage	↑ Roldan et al. (2007)	Clay	Vertisol
	No tillage (direct drilling)	↑↑ Melero et al. (2008)	Clay	Chromic Haploxeret
	No tillage	↑ Mina et al. (2008)	Sandy clay loam	–
	No tillage	– Mikanová et al. (2009)	Sandy clay loam	–
	No tillage (mouldboard ploughing)	↑ Lopez-Garrido et al. (2010)	–	Eutric Leptosol
	Reduced tillage (chisel ploughing)	↑ Ramos et al. (2011)	Clay loam	Hypercalcic Calcisol
Catalase	No tillage	↑↑ Ulrich et al. (2010)	Sandy loam	Stagnic Luvisol

For each enzymatic activity the referred biogeochemical cycle is reported. (↑, ↓ Increase or decrease of less than 100%; ↑↑, ↓↓ increase or decrease of more than 100%; – no effect)

7.3 PLFAs and Soil Carbon Sequestration Strategies

The determination of soil phospholipid fatty acids (PLFAs) is a powerful and still widely used method to assess the structure of viable microbial communities in soils. Phospholipids are at the basis of life itself, since they represent the structural skeleton of most living cells. The double layer of phospholipids found in most cells (exception

represented by Archaea, where tetraether lipids substitute phospholipids) allows the separation of living cells from the surrounding environment, and regulates, together with proteins, sterols and glycolipids, the exchanges between cells and their outside. Phospholipids are made up by a hydrophilic head constituted by a negatively charged phosphate group, usually substituted with a choline, and a hydrophobic tail, usually constituted by two fatty acids. These fatty acids belong to different classes, the most common ones being saturated, monounsaturated, polyunsaturated, branched, and cyclopropane (Fig. 7.1). As explained below, fatty acids composition of cell membranes differs from species to species (and thus can also be used for community structure assessments), and, within a single species, it is sensitive to a number of environmental parameters (e.g., temperature, nutrients, pollutants).

Bligh and Dyer (1959) published the first method for the identification and estimation of individual phospholipids in biological samples. The rationale of the method is based on isolation of the phospholipidic fraction, and removal from

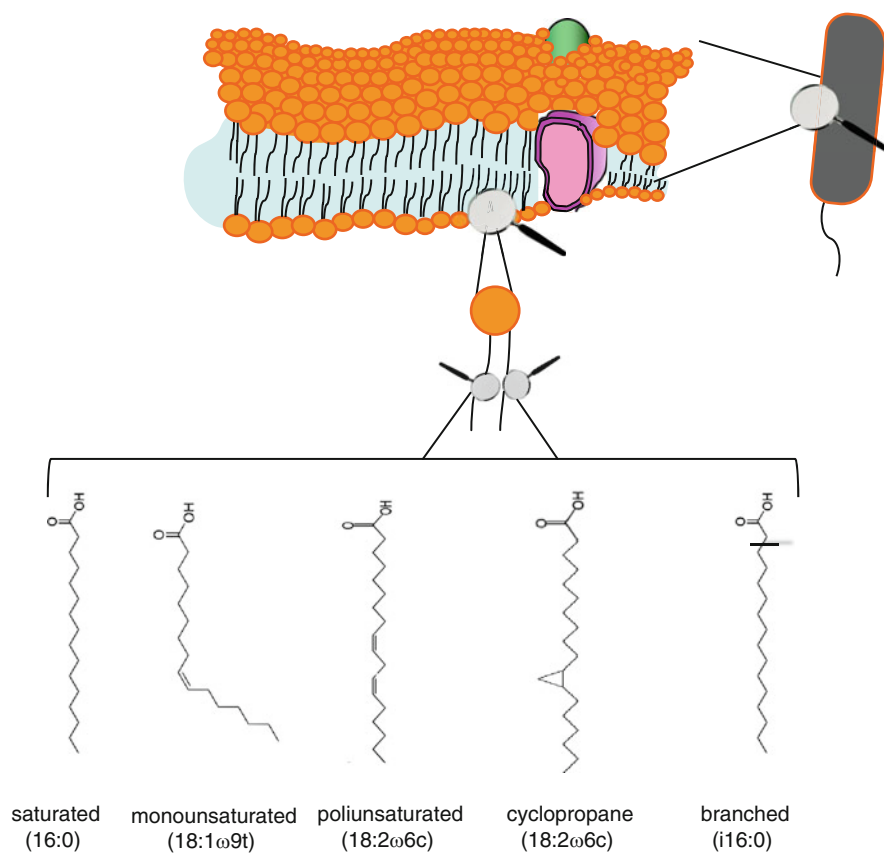


Fig. 7.1 Schematic representation of a microbial cell, its double layer phospholipidic membrane and examples of the most important classes of phospholipids fatty acids

phospholipids of single fatty acids by an alkaline hydrolysis. Then, a method was devised for the conversion of fatty acids in methyl esters and their easy determination by silica chromatographic analyses (Luddy et al. 1960). Marr and Ingraham (1962) assessed the composition of individual PLFAs in *Escherichia coli* cells grown at 10, 15, 20, 25, 30, 35, 40 and 43°C. They found that the increase of temperature resulted in an increase in hexadecanoic acid (16:0) and a decrease in unsaturated acids such as hexadecenoate (16:1). Furthermore, they found that the composition in fatty acids reflects the composition of the growth medium, but does not correlate with the culture growth stage. This was a milestone paper since it casted the basis of PLFAs use as ecological biomarkers of environmental conditions. Moreover, derived concepts such as the ratio between specific saturated and unsaturated fatty acids as index of specific conditions are still fundamental in the environmental extension of PLFA studies. PLFAs have been also used for the ecological assessment of microbial communities in marine and estuarine sediments (White et al. 1979), and, later, for the more complex soil microbial communities (Tunlid et al. 1985; Nichols et al. 1986; Vestal and White 1989).

Despite the introduction in later years of more advanced methods (especially those based on nucleotides), PLFAs analysis remains a widely used and useful method for the analyses of soil microbial communities. An enquire in SCOPUS using “soil*” and “PLFA*” as a search key among titles, keywords and abstracts gives a total of 819 papers between 1987 and today, with the number of papers per year almost constant in the last years (Table 7.1). Similarly to the case of enzymatic activities, Table 7.1 shows how tillage and organic fertilization represent only a part (around 10%) of the effects studied in the scientific literature.

A number of reasons make PLFAs still a very useful and informative method. First, fatty acids are easily degraded after cells death and, thus, their analyses give a snapshot on the total viable microbial communities. This is a full advantage, since in DNA-based analyses it is often difficult if not impossible to distinguish between living and dead cells. The second feature is related to the fact that specific PLFAs analysis (Table 7.4) can provide reliable information about microbial groups such as Gram-positive and Gram-negative bacteria, actinomycetes, fungi, protozoa, arbuscural mycorrhiza and total fungi (Zelles 1999; Bougnom et al. 2010).

Table 7.4 PLFA biomarkers and their use for the analysis of specific groups and conditions

Bacterial group/environmental condition	Biomarker PLFAs
Gram-positive bacteria	Sum of i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0
Gram-negative bacteria	Sum of cy17:0, cy19:0, 18:1ω9c, 16:1ω9c, 18:1ω9c, 15:1ω4c and 18:1ω7c
Arbuscural mycorrhiza	16:1ω5c
Protozoa	Sum of 20:2ω6,9,c, 20:3ω6,9,12c and 20:4ω6,9,12,15c
Actinomycetes	10Me16:0 and 10Me18:0
Fungal biomass	18:2ω6c and 18:1ω9
Total microbial biomass	16:0
Stress conditions	cy19:0/18:1ω7c ratio

Furthermore, some ratios between specific PLFAs have been proposed as indicators of stress conditions, and a numerical index of soil alteration based on PLFA has also been proposed (Puglisi et al. 2005).

A bibliographic search on PLFA values, as the one described above for enzymatic activities, is reported for the effects of organic amendments to soil (Table 7.5) and for those of soil tillage practices (Table 7.6). As it is indicated in each retrieved paper, aggregated PLFA data have been reported in order to refer to specific groups (i.e., actinobacteria, Gram-positive, Gram-negative, fungi and arbuscural mycorrhiza).

Five papers dealt with the effects of organic fertilizers on PLFAs (Table 7.5). The organic fertilizer additions were: composted red clover at 15 ton ha⁻¹ (Elfstrand et al. 2007a), farmyard manure at 4 ton of carbon ha⁻¹ (Elfstrand et al. 2007b), wood ash compost mixed with soil at 33% v:v ratio (Bougnom et al. 2010), compost at 10 ton ha⁻¹ (Treonis et al. 2010), and composted manure at 373 kg of nitrogen ha⁻¹ (Kong et al. 2011). Actinobacteria was the only microbial group that was not affected by organic fertilization, whereas significant changes in the community structure were found for all other groups. Specifically, fungi were always increased (five out of five studies), arbuscural mycorrhiza were increased in four studies and unaffected in one report, while total microorganisms (as estimated by the sum of all PLFAs) were significantly increased in four out of four studies.

Contrasting results about Gram-positive and Gram-negative bacteria were shown in these works. No significant differences for both groups were found in the study of Elfstrand et al. (2007b), while both groups were increased after the organic fertilization conducted by Treonis et al. (2010) and Bougnom et al. (2010). Finally, Kong et al. (2011) reported a decrease in both bacterial groups after addition of composted manure.

Five papers dealing with the effects of soil tillage on PLFAs have also been reviewed (Table 7.6). All these papers compared no tillage versus conventional tillage. No significant reductions on specific microbial groups were experimentally found, although results indicated an increasing trend in some cases. These findings were common for each microbial group, thus suggesting that effects of no tillage on microbial communities structure was quite site-specific, and most probably affected by general variables such as climate, soil type and cultivation. An exception was represented by Gram-positive bacteria, whose numbers were never affected by soil tillage treatments.

7.4 The MESCOSAGR Case Study

The MESCOSAGR project aimed at assessing the sustainability of methods for soil carbon sequestration (see Foreword) and their effects on soil physical, chemical, biological parameters as well as crop productivity. Two innovative methods have been compared with traditional and reduced tillage methods (see Chap. 1). The innovative methods were: the soil amendment with mature humified compost to promote a hydrophobic protection against microbial degradation of the more easily

Table 7.5 Analysis of literature info on the effects of organic fertilizers on soil PLFAs

Microbial group	Organic fertilizer applied	Effect	Soil texture	Soil taxonomy
Total microorganisms	Composted red clover (15 ton ha ⁻¹)	↑ Elfstrand et al. (2007a)	Silty clay loam	–
	Farmyard manure (4 ton C ha ⁻¹)	↑ Elfstrand et al. (2007b)	Clay loam	Eutric Cambisol
	Wood ash compost (33%)	↑↑ Bougnom et al. (2010)	Clay	–
	Compost (10 ton ha ⁻¹)	↑ Treonis et al. (2010)	Loamy sand	–
Actinobacteria	Composted manure (373 kg N ha ⁻¹)	- Kong et al. (2011)	Silt loam	Typic Xerorthent
	Compost (10 ton ha ⁻¹)	- Treonis et al. (2010)	Loamy sand	–
Gram-positive	Farmyard manure (4 ton C ha ⁻¹)	- Elfstrand et al. (2007b)	Clay loam	Eutric Cambisol
	Compost (10 ton ha ⁻¹)	↑ Treonis et al. (2010)	Loamy sand	–
	Wood ash compost (33%)	↑↑ Bougnom et al. (2010)	Clay	–
	Composted manure (373 kg N ha ⁻¹)	↓ Kong et al. (2011)	Silt loam	Typic Xerorthent
Gram-negative	Farmyard manure (4 ton C ha ⁻¹)	- Elfstrand et al. (2007b)	Clay loam	Eutric Cambisol
	Compost (10 ton ha ⁻¹)	↑ Treonis et al. (2010)	Loamy sand	–
	Wood ash compost (33%)	↑↑ Bougnom et al. (2010)	Clay	–
	Composted manure (373 kg N ha ⁻¹)	↓ Kong et al. (2011)	Silt loam	Typic Xerorthent
Fungi	Composted manure (373 kg N ha ⁻¹)	↑ Kong et al. (2011)	Silt loam	Typic Xerorthent
	Composted red clover (15 ton ha ⁻¹)	↑ Elfstrand et al. (2007a)	Silty clay loam	–
	Farmyard manure (4 ton C ha ⁻¹)	↑ Elfstrand et al. (2007b)	Clay loam	Eutric Cambisol
	Wood ash compost (33%)	↑↑ Bougnom et al. (2010)	Clay	–
Arbuscular mycorrhiza	Compost (10 ton ha ⁻¹)	↑↑ Treonis et al. (2010)	Loamy sand	–
	Composted red clover (15 ton ha ⁻¹)	- Elfstrand et al. (2007a)	Silty clay loam	–
	Farmyard manure (4 ton C ha ⁻¹)	↑ Elfstrand et al. (2007b)	Clay loam	Eutric Cambisol
	Wood ash compost (33%)	↑↑ Bougnom et al. (2010)	Clay	–
Arbuscular mycorrhiza	Compost (10 ton ha ⁻¹)	↑ Treonis et al. (2010)	Loamy sand	–
	Composted red clover (15 ton ha ⁻¹)	- Elfstrand et al. (2007a)	Silty clay loam	–
	Farmyard manure (4 ton C ha ⁻¹)	↑ Elfstrand et al. (2007b)	Clay loam	Eutric Cambisol
	Wood ash compost (33%)	↑↑ Bougnom et al. (2010)	Clay	–

↑, ↓ Increase or decrease of less than 100%; ↑↑, ↓↓ increase or decrease of more than 100%;
 - no effect

Table 7.6 Analysis of literature info on the effects of tillage on soil PLFAs

Microbial group	Tillage	Reduced/no tillage effects	Soil texture	Soil taxonomy
Total microorganisms	No tillage	↑ Chaer et al. (2009)	Loamy	Typic Fragiudults
	No tillage	↑ Helgason et al. (2010b)	–	–
Actinobacterial	No tillage	– Treonis et al. (2010)	Loamy sand	–
	No tillage	– Treonis et al. (2010)	Loamy sand	–
Gram-positive	No tillage	– Muruganandam et al. (2009)	Sandy clay loam	Typic Kanhapludult
	No tillage	– Helgason et al. (2010a)	–	–
Gram-negative	No tillage	– Treonis et al. (2010)	Loamy sand	–
	No tillage	↑ Chaer et al. (2009)	Loamy	Typic Fragiudults
	No tillage	– Muruganandam et al. (2009)	Sandy clay loam	Typic Kanhapludult
	No tillage	– Helgason et al. (2010a)	–	–
Fungi	No tillage	– Treonis et al. (2010)	Loamy sand	–
	No tillage	↑ Muruganandam et al. (2009)	Sandy clay loam	Typic Kanhapludult
	No tillage	↑ Helgason et al. (2010b)	–	–
	No tillage	– Treonis et al. (2010)	Loamy sand	–
Arbuscular mycorrhiza	No tillage	↑ Helgason et al. (2010a)	–	–
	No tillage	– Treonis et al. (2010)	Loamy sand	–
	No tillage	↑ van Groenigen et al. (2010)	Sandy loam	Haplic Luvisol
Stress biomarker cy19:0/18:1ω7c	No tillage	↑ Chaer et al. (2009)	Loamy	Typic Fragiudults
	No tillage	– Helgason et al. (2010a)	–	–

↑, ↓ Increase or decrease of less than 100%; ↑↑, ↓↓ Increase or decrease of more than 100%;
 – No effect

degradable soil organic matter fraction, and an in situ photo-polymerization of soil organic matter catalyzed by a water-soluble iron–porphyrin catalyst spread on soil.

The analytical methods described above (enzymatic activities and PLFA analyses) have been applied in order to assess any possible effects of the tested practices on the biochemical quality of soils as assessed by measures of the activity (enzymatic analyses) and structure (PLFAs) of soil microbial communities.

Experiments were conducted for three consecutive years in the four different Italian locations described in the other chapters: Napoli, Torino, Piacenza and Potenza. The following treatments have been compared at each site:

- MIN: minimum tillage and mineral fertilization with urea (130 kg N ha⁻¹)
- COM2: second rate of compost (20 ton ha⁻¹, corresponding to 260 kg N ha⁻¹)
- TRA: traditional ploughing and mineral fertilization with urea (130 kg N ha⁻¹)
- CAT: catalyst treatment (1 g m⁻² of catalyst porphyrins, traditional ploughing and mineral fertilization with urea at 130 kg N ha⁻¹)
- No-CAT: no catalyst treatment

In the Piacenza site, all treatments were under maize cropping. In the Napoli and Torino sites, the MIN, COM2 and TRA treatments were for soil under maize,

whereas CAT and No-CAT treatments were adopted under wheat. Finally, in the Potenza site, a specific comparison between TRA, COM2 and COM1 (equal to COM2 but with half compost rate) treatments were conducted under sorghum. Specific details about soil and climatic conditions in each site are reported in Chap. 3.

At each location, soil samples were collected after harvesting every year for three consecutive years (2006, 2007 and 2008). All samples have been sieved at 2 mm immediately after sampling and stored at 4°C for maximum 2 months until analysed.

Four enzymatic activities were determined. β -Glucosidase (E.C. 3.2.1.21) and phosphatase (E.C. 3.1.2.1) were analysed according to Eivazi and Tabatabai (1990) and Sannino and Gianfreda (2001), using, respectively, *p*-nitrophenyl- β -D-glucoside and *p*-nitrophenylphosphate as substrates; urease (E.C. 3.5.1.5) and invertase (E.C. 3.2.1.26) were determined according to Kandeler and Gerber (1988) and Sannino and Gianfreda (2001), using urea and saccharose as substrates. Enzymatic activities were expressed as μmol (for β -glucosidase, phosphatase and invertase) or μg (for urease) of substrate hydrolyzed per hour and per g of dry soil.

The analysis of PLFAs was conducted according to the original Bligh and Dyer (1959) method, as modified by Ibekwe and Kennedy (1998). The lipidic phase was extracted from soil by a mixture of methanol, dichloromethane and sodium bromide. Phospholipids were separated from glycerolipids and neutralipids on columns, and transesterified by saponification. The obtained fatty acid methyl esters (FAME) were then determined with an Agilent 5973N GC, equipped with a 30 m $\times$ 0.25 mm ID cross-linked methyl silicone (0.25 μm film thickness) HP-5-MS capillary column. A splitless injection was employed (injector at 280°C) and the oven was held at 70°C for 2 min after injection. The oven temperature was then ramped to 160°C at 40°C min⁻¹, and again to 280°C at 3°C min⁻¹, using helium as carrier gas (1 ml min⁻¹). The run lasted for 40 min, long enough to allow column elution of fatty acids up to 26 carbons. Each PLFA was identified by comparing both retention time and mass spectra with analytical standards. Concentrations were quantified from peak areas of representative ions injected every five samples, after linear interpolation of standards at concentrations increasing from 0.1 to 15 mg kg⁻¹. The concentration of each PLFA was normalized to the 19:0 fatty acid, used as internal standard.

Results for the last year of experimentation are reported here and discussed in order to provide information about the cumulative effects of 3 years of C sequestration strategies. Statistical analyses were carried out by SAS software (1995). A mixed model analysis of variance (ANOVA, PROC MIXED, SAS) was applied. In a mixed model ANOVA, one or more factors are defined as *fixed*, and one or more as *random*. A *fixed* factor has levels that are determined by the operator, while a *random* factor has levels that are chosen randomly from the population of all possible levels (Sit 1995). A main effect to be detected and evaluated is normally a *fixed* factor, while an effect that contributes to the data spreading is a *random* one. In this work, the classification variable TREAT, that is the effect of soil C sequestration strategy (classification levels MIN, TRA, COM1, COM2, CAT and

No-CAT) has been treated as a *fixed* variable, whereas SITE (classification levels Piacenza, Torino, Napoli and Potenza) was assumed as a random one. The interaction TREAT*SITE was *random* as well. Significant effects were confirmed and further investigated for specific differences between class levels by Tukey's test for comparison of means by assessing the effect of treatment separately per each site. The ANOVA results are reported in Table 7.7 for four soil enzymatic activities and for soil microbial groups estimated by PLFAs analysis (total microorganisms, Gram-positive, Gram-negative, protozoa, fungi, actinomycetes).

Both SITE and TREAT*SITE effects were significant for β -glucosidase, whereas TREAT*SITE was the only significant effect for both phosphatase and urease (Table 7.7). Finally, both SITE and TREAT*SITE effects were significant for invertase. The SITE effect was confirmed by the Tukey's test, with data for Piacenza and Potenza being significantly larger than for Napoli and Torino.

Table 7.7 Application of a mixed model analysis of variance to enzymatic activities and PLFA data from the different sites of MESCOSAGR

	TREAT	SITE	TREAT*SITE
β -Glucosidase	0.84 ^{ns}	0.65**	7.78***
Phosphatase	0.14 ^{ns}	1.85 ^{ns}	3.41**
Urease	1.29 ^{ns}	3.76 ^{ns}	3.72**
Invertase	0.86 ^{ns}	14.19**	6.09***
Total microorganisms	1.31 ^{ns}	1.33 ^{ns}	0.76 ^{ns}
Gram-positive	0.66	3.61 ^{ns}	2.37*
Gram-negative	1.42 ^{ns}	1.29 ^{ns}	1.90 ^{ns}
Protozoa	1.23 ^{ns}	0.82 ^{ns}	0.84 ^{ns}
Fungi	0.19 ^{ns}	0.19 ^{ns}	2.75*
Actinomycetes	0.77 ^{ns}	4.95*	1.93 ^{ns}

Per each effect *F* values are reported. TREAT is the effect of soil C sequestration strategy (classification levels MIN, TRA, COM1, COM2, CAT and NO-CAT), SITE represents the location (classification levels Piacenza, Torino, Napoli and Potenza) and TREAT*SITE their interaction

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ^{ns}nonsignificant

Specific differences per each enzymatic activity were further analysed by assessing, through the Tukey's test, the effect of treatments separately at each sampling location. For β -glucosidase, MIN values were found significantly larger than all other treatments in Piacenza, while the enzyme's activity of MIN was still greater than TRA in the Napoli site (Fig. 7.2). Again, MIN provided larger phosphatase (Fig. 7.3) and urease values (Fig. 7.4) than other treatments in Piacenza, whereas no significant differences were instead found among treatments for the other sites. Similar findings were also shown for invertase in Piacenza (Fig. 7.5), while both CAT and No-CAT provided larger enzyme values than TRA and COM2 treatments in Torino. However, it should be highlighted that in Torino, differently from Piacenza, the CAT and No-CAT trials were conducted under wheat, while MIN, TRA and COM2 were under maize.

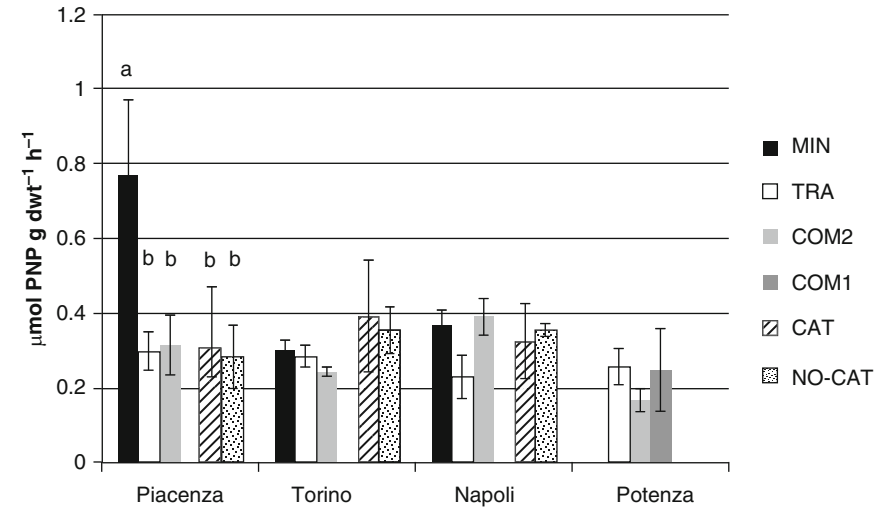


Fig. 7.2 β -Glucosidase activity in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

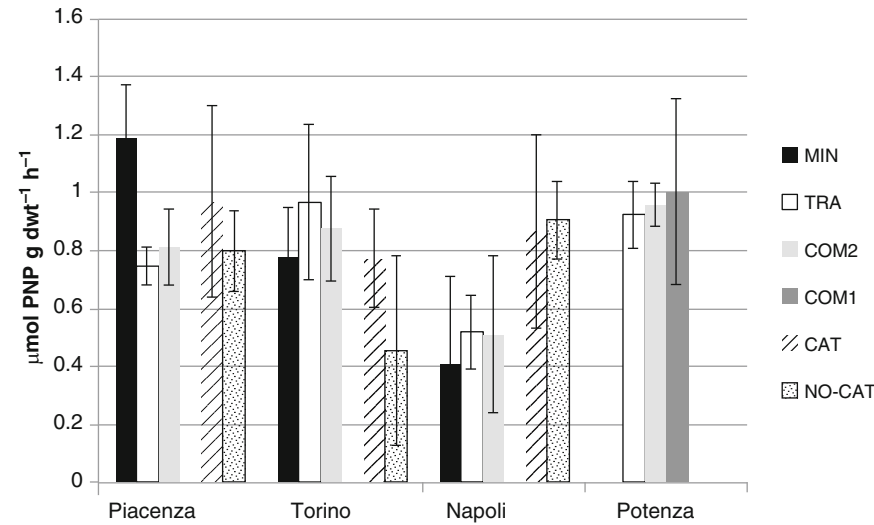


Fig. 7.3 Phosphatase activity in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

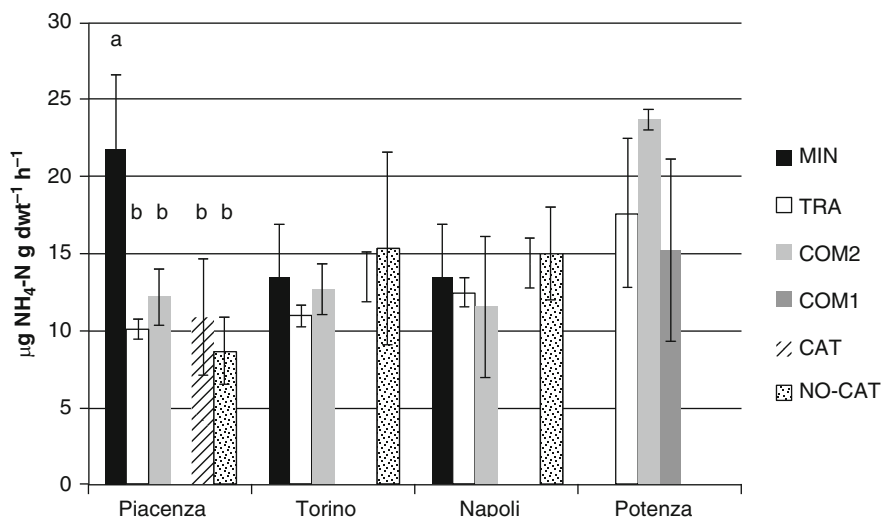


Fig. 7.4 Urease activity in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

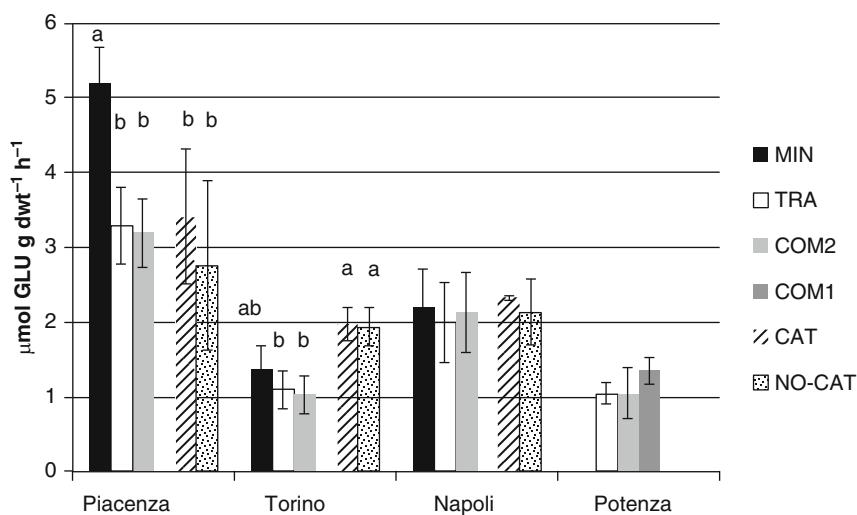


Fig. 7.5 Invertase activity in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

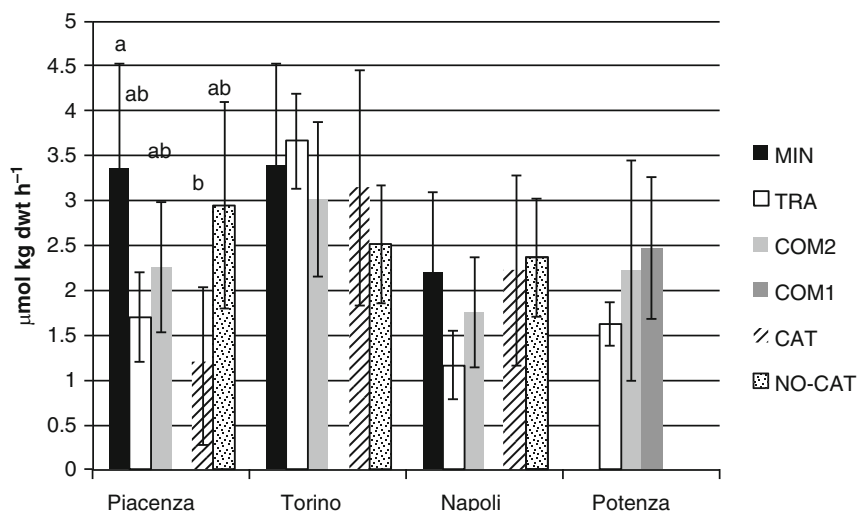


Fig. 7.6 PLFAs estimation of Gram-positive bacteria in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

Microbial groups, as estimated by specific PLFAs (identified according to Table 7.4), are also reported in Table 7.7. The statistical elaboration of the PLFA findings indicates that there were no significant effects among treatments in the different sites for total microorganisms, Gram-negative bacteria and protozoa (data not shown). Conversely, it was found that the relation TREAT*SITE was significant for Gram-positive bacteria (Fig. 7.6) and fungi (Fig. 7.7), while for actinomycetes (Fig. 7.8) the SITE variable had a significant effect. Only data for these three groups are presented and discussed here.

According to the Tukey's test for comparison of means that was conducted separately per each site, Gram-positive bacteria (Fig. 7.6) were significantly larger in Piacenza in the MIN treatment as compared to COM2, but no other differences were found among all other treatments and in the other sites. For fungi (Fig. 7.7), the only significant difference was in the Torino site, where the CAT treatment produced a larger level than for MIN, TRA and COM2. However, since no significant difference was found between CAT and No-CAT, the latter finding in Torino is to be attributed more probably to a plant (wheat for CAT and No-CAT and maize for all other treatments) rather than to a treatment effect. Finally, for actinomycetes (Fig. 7.8) the situation was very similar to that for Gram-positive bacteria, with significantly larger values in the Piacenza site for MIN than for COM2, though no other differences were shown among all other treatments and in the other sites.

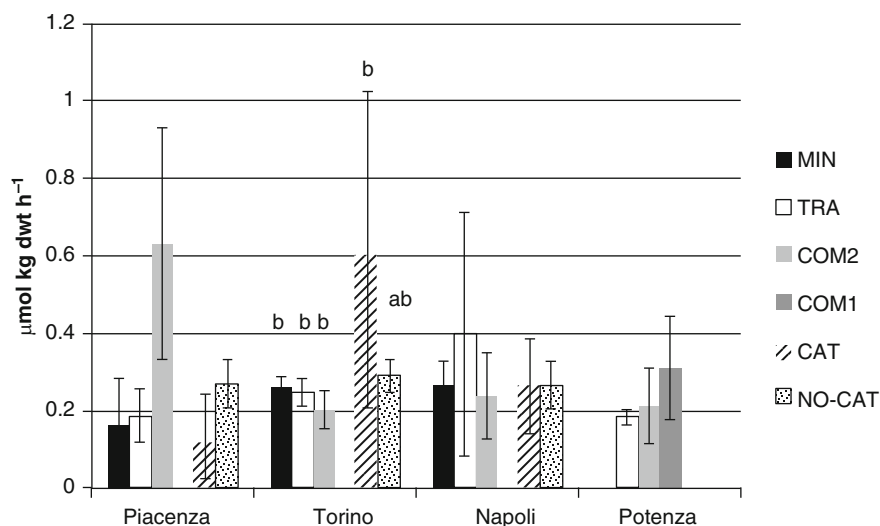


Fig. 7.7 PLFAs estimation of fungi in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

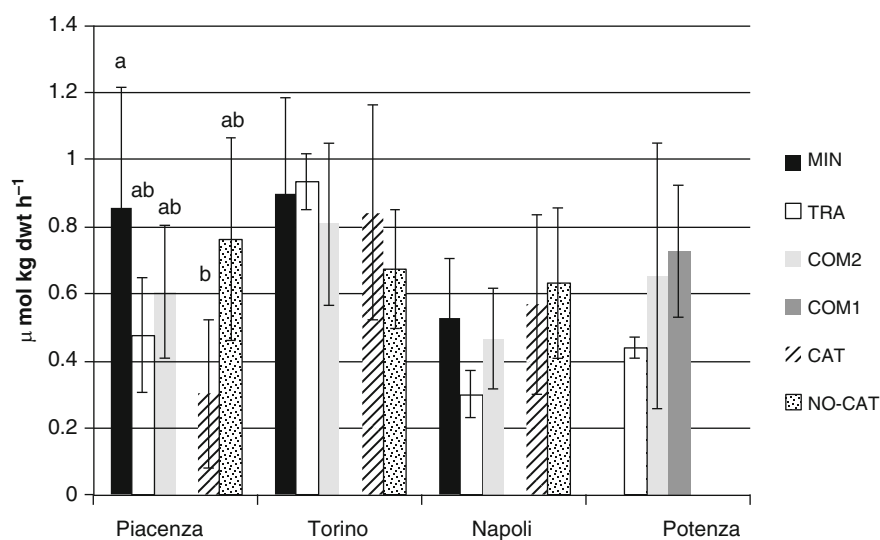


Fig. 7.8 PLFAs estimation of actinomycetes in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

7.5 Discussion of Results

We have used both bibliographic and original data from the MESCOSAGR project to compare the effects of C sequestration methods on selected biochemical indicators of soil quality, such as enzymatic activities and PLFAs. We found (Tables 7.1–7.6) that much information is available in literature in response to soil treatments such as addition of humified composted matter and different tillage systems, but it has never been summarized and critically evaluated. In the case of the carbon sequestration method based on the catalyst-assisted in situ photo-polymerization of SOM, there are no information yet available on the soil biological responses, and, thus, our results are original, together with those also reported in Chap. 6.

According to literature, some trends were identified for the responses of enzymatic activities to compost addition (Table 7.2), and were even more definite for the responses to tillage (Table 7.3). Based on reports regarding 12 enzymatic activities, these were in most cases induced after addition of different types of compost and manure, at different rates and in different soil types. In some specific cases, no significant differences or inhibitions were found, usually because of presence of contaminants or other compounds, or by the application of too small rates. In response to tillage treatments, the enzymatic activities reported were even clearer in showing a general trend of larger values in no tilled plots (Table 7.3). However, a number of other works indicated that soil organic amendment or reduced tillage had no effects on most enzymatic activities or even resulted in their inhibition. Other soil parameters or management practices (e.g., pest control strategies) were also accounted to play a role on soil biological responses (Omar and Abdel-Sater 2001).

For the MESCOSAGR project, the effects of soil compost amendment and tillage practices were followed on four selected enzymatic activities (β -glucosidase, phosphatase, urease and invertase). The soil treatments were found to hardly have an influence on these enzymes. Specifically, it was found that only the minimum tillage treatment had an effect on urease, β -glucosidase and invertase, and only in the Piacenza site. Neither compost additions nor the biomimetic catalyst spread resulted in significant changes of the four enzymatic activities.

The determination of PLFA values in soil provides indications on the content and changes of different microbial groups (Table 7.3). The soil PLFA patterns were generally altered by compost additions in soil or changes in tillage practices. These alterations lacked, however, a consistent direction, as some microbial groups were found to be either enhanced or reduced, or not affected at all. This variability in literature data was confirmed by results obtained at the third year of experimentation within the MESCOSAGR field trials. These findings showed that some microbial groups (Gram-positive, fungi, actinomycetes) changed according to field site and/or soil treatments, while other showed almost constant levels across sites.

A comparison of the third-year values with those obtained after the first experimentation year (data not shown here) suggests that the content of enzymatic activities and PLFAs in MESCOSAGR soils increased after 3 years of treatments,

but not in all cases. Specifically, it was found an increase in Piacenza for invertase and urease for the MIN treatment, while enzymatic activities were relatively constant in the other sites, if not even progressively reduced in some cases. Analyses of PLFAs data showed some changes in the global pattern with time, though the trends reported in Table 7.7 and in Figs. 7.6–7.8 were quite constant. These outcomes confirm the variability of these parameters in time, and the importance of carrying out experimentation for some consecutive years in order to identify and confirm specific trends.

7.6 Conclusions

The results illustrated in this chapter allow drawing some general conclusions. Enzymatic activities are usually correlated with soil quality, and, if not induced by disturbing agents (e.g., organic pollutants stimulating soil microflora activity), their increase is usually positive, as it indicates an efficient microbial recycling of essential plant nutrients.

A more complex issue is the relation between CO₂ emission from soils (the main study of the MESCOSAGR project) and the biological parameters dealt with here. An increase in enzymatic activities related to total microbial activity (dehydrogenase, FDA, catalase) will most probably result in larger CO₂ emissions, although other factors such as organic carbon quantity and quality should be also considered. On the other hand, an increase in activities of enzymes involved in the biogeochemical cycles of phosphorus, sulfur and nitrogen do not necessary result in an increased CO₂ emission. For example, in the case of phosphatase, it was found that this enzyme is more correlated with environmental availability of P than to decomposition rates, and that there is no relationship between CO₂ efflux and its activity, at least in litter environments (Johnson et al. 2010). This was an important result and should be confirmed for other enzymatic activities, since it may derive that C sequestration methods can be applied to increase the biochemical quality of soils without affecting, or even reducing, CO₂ emission. The relationship with other greenhouse gases should be also considered, especially in the case of N-related enzymatic activities which may affect N₂O fluxes. However, our results within the MESCOSAGR project have shown that in four different Italian locations the adopted field methods for C sequestration did not significantly changed an important component of soil biochemical quality, such as soil enzymatic activities.

Upon a stimulation of soil microbial activities by carbon sequestration practices, the composition of microbial communities should also be affected. This was verified by our PLFA analyses, which also showed that microbial communities were shifted differently than for enzymatic activities. This discrepancy implied that values on enzymatic activities provide soil functional quality, while PLFAs (especially if analysed with a taxonomical approach) account for structural soil composition. Thus, it may be expected that a specific change in a soil function (e.g., an increase in phosphatase activity) can be related to a number of different changes at

taxonomical level. This consideration highlights the importance of including both structural and functional measurements, when verifying the effects of soil treatments on soil microorganisms. Another conclusion of our approach is that changes in microbial communities should not be negatively considered, but they should be evaluated in the wider context of soil ecological functions.

Finally, it must be pointed out that the use of the biomimetic catalyst as a practice to sequester C in soil (the CAT treatment) through photo-polymerization of SOM, failed to bring about any change in the enzymatic activities in four different Italian field site under both maize and wheat cropping. As for PLFAs, the CAT treatment produced only a specific increase in fungal population of the Torino site.

7.7 Recommendations for Future Experimentation

Our MESCOSAGR results showed that the applied methods for soil carbon sequestration had no negative effects on the soil biochemical quality. We found no effects in some cases, and even positive effects in many other cases. This was in line with the literature review illustrated above. This work also confirms the importance of linking structural with functional measurements, when assessing the response of soil microbial communities to any experimental factor.

It is recommended that future investigations will continue to apply well-established methods such as enzymatic activities and PLFA analyses for evaluation of soil biochemical quality. However, they will have to be increasingly supported by novel advanced techniques, such as meta-genomics and meta-transcriptomics, in order to reach more valid evidence on the changes induced by soil treatments. Both PLFA and enzymatic methods will be useful as benchmarks for future advanced methods, since they provide easily interpretable information capable to correctly interpret the large amount of data that the new technologies will provide.

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Chapter 8

Biological and Biotechnological Evaluation of Carbon Dynamics in Field Experiments

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Abstract Bacteria and fungi play a key role in promoting soil organic matter (SOM) turn over and consequent nutrient availability to plants uptake. During SOM degradation, they contribute to transform highly complex biomolecules to smaller compounds, which are either immobilized by soil microflora or self-associated in humified and microbially stable superstructures. These processes rapidly occur in the rhizosphere where soil adheres to plant roots and microbial populations are more abundant and active than in bulk soil. Despite the difficulties in determining the composition of soil microbial communities, their genetic and functional diversities are fundamental to maintain soil quality and productivity, even under environmental stress or alteration. Within the national MESCOSAGR project, we provided indications on the composition and diversity of bacterial communities in soils subjected to carbon sequestration treatments. Nucleic acids were extracted from rhizosphere and bulk soils, purified and amplified by polymerase chain reaction (PCR), targeting a conserved region of 16S rRNA gene. Amplicons were separated by denaturing gradient gel electrophoresis (DGGE) and cluster analysis of relative electrophoretic profiles was used to evaluate the diversity of bacteria communities in soils under different soil management practices. The PCR products have also been cloned and sequenced in order to identify and characterize the microbial groups and species which populated the experimental soils. Our results, relative to field trials of the first 2 years and three experimental sites, indicate that the application of different molecular approaches contribute to reach an advanced characterization of structure and diversity of soil bacteria, as well as an appraisal of their variation, as a consequence of specific soil management practices. In particular, it appears that only the amendments with mature compost had a significant effect on the soil microbial communities, while

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other soil treatments such as that with the iron–porphyrin biomimetic catalyst did not have any effect.

8.1 Introduction

Soil microorganisms are determinant in functioning and stability of soil ecosystem because of their influence in nutrient cycles and energy fluxes (Wertz et al. 2007). In particular, bacteria and fungi promote organic matter turn over and the consequent nutrient availability for mineral nutrition of plants (Ladd et al. 1996). In fact, in the degradation step of soil organic matter (SOM), they contribute to the decomposition of highly complex compounds to more simply compounds that, in turn, are immobilized by soil microflora or are aggregated in the humification process, giving rise to compounds recalcitrant to further microbial degradation (Smith et al. 1993).

The dynamics of the organic matter degradation depends on the nature and the quality of decomposing materials. Hodge et al. (2000) demonstrated that the amount of decomposed organic matter depends on C/N ratio of the decomposing material more than on the C and N needs of soil microorganisms. Compound with a low C/N ratio are easily degradable and rapidly incorporated by microbial biomass while compounds with a higher C/N ratio typically contain polymers such as lignin and polyphenols that are slowly degraded and only partially assimilated by soil microflora (Nicolardot et al. 2001). Furthermore, poorly degradable compounds might induce a kind of selection among microbial communities by favoring species with low metabolism (Lagomarsino et al. 2006). Repeated applications of manure for 10 years favored development of different microbial communities from those present in soils amended with chemical fertilizers (Toyota and Kuninaga 2006). Other authors demonstrated that microbial metabolites largely contributed the stable pool of C (Kiem and Kögel-Knabner 2003; Kindler et al. 2006; von Lützow et al. 2006; Simpson et al. 2007).

An important role is attributed to the rhizosphere, that is the soil adhered to plant roots, and where microbial populations are more abundant and active than in bulk soil (Curl and Truelove 1986). Long-term effects of rhizodeposition on soil C turnover have been proposed as a means to contrast greenhouse effects (Smit et al. 1997; Paustian et al. 1998). Experiments under laboratory scale conditions (Kuikman et al. 1990a, b; Hassink et al. 1993; Foereid and Yearsley 2004) showed an increased C sequestration due to rhizosphere microorganisms in grazing grounds.

Genetic and functional diversity of soil microbial communities are fundamental to keep high levels of soil quality and productivity, even under stress conditions and in altered environments. On the other hand, it is very difficult to determine the composition of microbial communities in the very complex and dynamic soil biological system (Nannipieri et al. 2003).

For a complete view of soil environment conditions, it is important to apply analytical methods capable to investigate the diversity of microbiota, their function and distribution in soil (Hill et al. 2000). Size and composition of microbial

communities can be obtained by extraction, quantification and characterization of biomarkers, which are molecules highly specific for microbial groups and species. The qualitative and quantitative determinations of biomarkers allow the investigation of the whole communities, including those species that are either poorly or not at all cultivable under laboratory conditions. Many cell components can be assumed as biomarkers. In particular, nucleic acids (DNA, RNA) give useful information on the structure and phylogenetic composition of soil microorganisms (Griffiths et al. 2000).

Since bacteria can be found within soil aggregates or on their surface, the capacity to separate bacterial cells from soil particles is a key step in the study of biodiversity (Trevors 1998). There are two basic methods to extract nucleic acids from soil: a direct and an indirect method. The direct method was applied for the first time by Torsvik (1980), and consists of a preliminary desorption of bacterial cells from soil particles, followed by their lysis and further purification of the released nucleic acids. The direct method was proposed for the first time by Ogram et al. (1987). It entails the direct lysis of bacterial cells without a previous separation from soil and subsequent purification steps. This method has been successfully applied to investigate extracellular nucleic acids naturally present in soil microcosms. In both methods, cell lysis can be performed by a combination of mechanical (glass bead beater), chemical (detergents, NaOH) and enzymatic (lysozyme, protease K) methods. The efficient cell lysis and nucleic acids extraction, as well as the purity and size of extracted nucleic acids represent the major critical steps for further molecular characterizations (Ogram 2000).

Among the various molecular approaches, the more useful method for qualitative/quantitative characterization of microbial communities comprises the polymerase chain reaction (PCR) for prokaryotic genes coding the small subunit of ribosomal RNA (16SrDNA) (Ward et al. 1992). PCR is a polymerization reaction catalyzed by a thermo-stable DNA polymerase (Saiki et al. 1988) that allows the selective enrichment, of small target genome regions, without cloning into vectors/host cells. The characterization of microbial communities by amplified genomic sequences coding for ribosomal RNA can be carried out by denaturing gradient gel electrophoresis (DGGE) (Muyzer et al. 1993), a technique that gives information at the level of microbial groups more than at that of species (Nannipieri et al. 2003). By PCR, amplicons of the same size but even slightly differing in nucleotide sequence can be separated according to their melting temperature. During electrophoresis, each fragment remains as double strand until an exact concentration of denaturing chemicals (a linear gradient of urea and formamide at 60°C) is reached in the gel run and a denaturation occurs, thus causing conformational changes and loss of electrophoretic mobility (Heuer and Smalla 1997). The presence of a 40–45 GC stretch (GC-clamp), within the sequence of one of the primers used to amplify the target region, increases the melting point of the amplified fragments, thus avoiding a complete denaturation of amplicons and enhancing the fragments separation capacity up to 500 bp (Muyzer et al. 1993). The 16SrDNA PCR/DGGE method produces electrophoretic profiles in which the number of bands corresponds to the richness of bacterial species (at least the more representative), while the band

intensities indicate the relative abundance of species. Moreover, a computational analysis of electrophoretic profiles allows the comparison among different samples and the build-up of phylogenetic trees. The amplification of DNA or RNA leads to discriminate either the whole bacterial population or the metabolically active microbiota, respectively (Prosser 2002).

A better comprehension of the relationship existing between microbial diversity and soil functions requires the use of high-resolution techniques (Nannipieri et al. 2003), being the cloning/sequencing of genomic regions undoubtedly one of them. Once again, the main target is represented by highly conserved regions of 16S rDNA that, after PCR amplification, can be easily cloned to give libraries, whose sequencing are representative of whole communities (Britschgi and Giovannoni 1991). Alternatively, PCR amplicons can be directly sequenced by the very recent pyro-sequencing approach (Acosta-Martínez et al. 2008). The matching of nucleotide sequences with databases such as NCBI GenBank or Ribosomal Database Project allows to establish a phylogenetic taxonomy of microbial species in different soil environments, as well as to compare their diversity, distribution, and abundance in soils (Maidak et al. 1997; Prosser 2002). By this approach, Curtis et al. (2002) showed that a gram of soil may contain up to 7,000 different bacteria species, that is the same amount estimated by traditional methods (Torsvik et al. 2002). Nevertheless, the definition of the number of sequences which may be adequate to represent a soil microbial diversity is still under debate.

Within the national MESCOSAGR project (see the Foreword and Chap. 3), the aim of our contribution was to provide indications about composition/diversity of bacterial communities of soils treated with carbon sequestration methods. Nucleic acids (DNA and RNA) have been extracted and purified by rhizosphere and bulk soil of three experimental fields and amplified by PCR targeting a conserved region of 16S rRNA gene. Amplicons have been separated by DGGE and cluster analysis of electrophoretic profiles has been used to evaluate the diversity of bacterial communities in soils under different soil management practices. PCR products have also been cloned and sequenced to identify microbial groups and species inhabiting the experimental soils.

8.2 Materials and Methods

8.2.1 *Experimental Setup*

Bacteria diversity has been investigated in soils collected from the experimental sites of Torino, Napoli, and Piacenza (see Chap. 3) where the following soil management practices were applied for maize cropping:

COM: traditional tillage (35 cm) and organic matter amendment consisting of 20 t/ha of compost and corresponding to 260 kg N/ha.

TRA: traditional tillage (35 cm) and chemical fertilization corresponding to 260 kg N/ha.

MIN: minimal tillage (10 cm) and chemical fertilization as for TRA; and for wheat cropping.

CAT: traditional tillage (35 cm) and spread of water soluble iron–porphyrin as catalyst for the in situ photo-oxidative polymerization of SOM (see Chaps. 1 and 4). Soil surface was amended before seeding by 1 g iron–porphyrin (dissolved in 5–10 l water)/m².

NoCAT: traditional tillage (35 cm) without any catalyst amendment.

The experimental design was at randomized blocks with either four replicates (TRA/MIN/COM) or three replicates (CAT/NoCAT). Each plot was sampled for both bulk and rhizospheric soil.

Rhizosphere soil was obtained by hand scrubbing, in sterile conditions, the 2 mm portion of soil tightly adhered to the roots of three plants per plot and pooling the collected soil in one sample per plot. Five subsamples of bulk soil per plot were collected at 35 cm depth, and pooled on site. Soil samples were sieved at 2 mm and stored at -80°C for RNA extraction and at -20°C for DNA extraction. The sequence of operations to extract both DNA and RNA from soils and obtain meaningful information from the nucleic acids on the effects of soil treatments on soil biodiversity are listed in Fig. 8.1.

8.2.2 Extraction and Purification of Nucleic Acids

Total DNA was extracted adopting the direct lysis method by using the FastPrep[®] System, a bead beater that allows to desorb microbial cells from soil particles and provides a large lysis yield. In combination with the commercial FastSPIN[®] kit

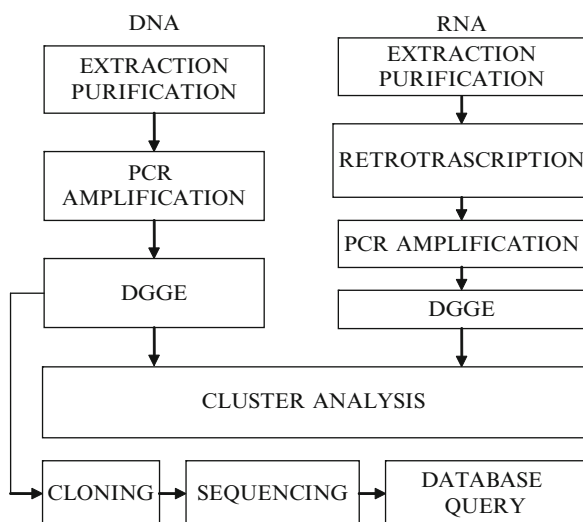


Fig. 8.1 Flow sheet of employed molecular techniques

for Soil (MoBio) this method gives a highly purified DNA from small amount (0.5 g) of soil.

One milliliter of sodium phosphate buffer and 0.1 ml lysis buffer were added to 0.5 g of soil in a lysing matrix tube. The soil slurry was homogenized for 30 s at 5.5 m/s, centrifuged at $14,000 \times g$ for 30 s, and the supernatant transferred to a 2.0 ml microcentrifuge tube. 0.25 ml of protein precipitation solution was added and the tube shaken ten times by hand before centrifuging at $14,000 \times g$ for 5 min to precipitate a protein pellet. The supernatant was transferred to a clean microcentrifuge tube in which 1 ml of binding matrix was added to bind DNA. After the setting of silica matrix for 3 min, about half of the supernatant was carefully removed, while the remaining part of the supernatant was resuspended in the binding matrix and then transferred to a SPIN filter to be centrifuged at $14,000 \times g$ for 1 min. SPIN filter was washed twice with 0.5 ml SEWS-M, air dried for 5 min and the DNA eluted with 0.1 ml DNase free water.

A direct lysis method was also adopted to extract RNA, by using the commercial RNA PowerSoil™ Total RNA Isolation (MoBIO). Two grams of soil were added to a 15 ml Bead Tube. 3.5 ml of a mix of bead/lysis solutions were added to the tube and this was vortexed for 5 min before adding 3.5 ml of phenol:chloroform:isoamyl alcohol mixture (25:24:1, pH 7.0) and shaken again by vortex for 10 min. Water phase was obtained after centrifuging at $2,500 \times g$ for 10 min and DNA ethanol precipitated, purified by a RNA Capture Column, eluted with 0.1 ml RNase free water and stored at -80°C before use.

8.2.3 Characterization of Extracted Nucleic Acids

DNA and RNA concentration was determined spectrophotometrically at 260 nm by the UV/visible NanoDrop® ND-1000, a spectrophotometer that used very low amounts of nucleic acid solution (1 μl) and concomitantly enabled to determine the quality indices A_{260}/A_{230} and A_{260}/A_{280} . Electrophoresis of nucleic acids on ethidium bromide stained 0.7% agarose gel was used to evaluate the average molecular weight (>10 kb), the presence of degradation smear for DNA, and the presence of the two major rRNA bands (0.9 and 1.5 kb for 16S and 23S rRNA, respectively).

8.2.4 DNase Digestion and Retrotranscription of RNA

Extracted RNA was digested with DNase to eliminate DNA traces eventually coextracted. Because single strand RNA does not constitute a valid template for PCR, RNA was retro transcribed to obtain the cDNA–RNA hybrid by using 100 U of the MMLV-RT enzyme and the RETROscript® kit (Ambion®), according to manufacturer' instructions.

8.2.5 PCR Amplification

Both DNA and cDNA–RNA hybrid extracted and purified from soil samples were amplified by PCR of conserved regions of the gene coding for the small subunit of ribosomal RNA. Eubacterial communities were investigated by using the 968F–1401R primers (Heuer and Smalla 1997), targeting the V6–V8 region of the 16S rDNA that, in *Escherichia coli*, spans between the 968 and 1,401 positions of the gene, and amplifying a fragment of about 450 bp (Brosius et al. 1978). In particular, the forward 968F primer was modified to contain a GC-clamp on the 5' end, a 40 G and C stretch that increases the melting temperature of the amplicons, thus avoiding their complete denaturation under denaturing gel electrophoresis (Muyzer et al. 1993). Reaction mix contained 20 ng DNA template, 3 U of Taq polymerase (Euroclone), 50 pmol of each of the two primers, 10 nmol each of dNTPs, 2.5 mM MgCl₂, in a buffered final volume of 50 µl.

Amplification conditions were as follows:

3 min at 95°C (denaturing step)	
30 s at 95°C	} (35 cycles)
30 s at 56°C	
30 s at 72°C	
10 min at 72°C (final elongation step)	

PCR fragments were checked by electrophoresis on ethidium bromide (0.5 µg/ml) stained 1.5% agarose gel.

8.2.6 Denaturing Gradient Gel Electrophoresis

In order to load the same amounts of amplicons onto gels free of unincorporated dNTPs and primers, PCR products were previously purified by the UltraClean™ PCR Clean-up Kit and quantitatively assayed by the NanoDrop ND-1000 spectrophotometer.

DGGE was performed by using the DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). 400 ng of PCR amplicons were loaded onto 6% (w/v) acrilamide (acrylamide, *N,N'*-methylenebisacrylamide, p/p, 37.5:1) vertical gel, with a “top-filling” linear gradient (that is, increasing denaturing conditions from the top to the bottom of the gel) ranging from 45 to 60% (100% corresponds to 7M urea – 40% v/v deionized formamide). Electrophoresis was performed for 15 h at 75 V 60°C in 1X TAE buffer (40 mM Tris base, 20 mM acetic acid, 1 mM EDTA, pH 8.3). Gels were stained in SYBR green I (Fluka), a nucleic acid gel stain that, once irradiated by UV lamps, emits fluorescent light at 520 nm. In order to compare gels run separately, even in the presence of a

large number of samples, a marker consisting of amplicons relative to the 16S rDNA of *Lactobacillus sanfranciscensis* and to the β subunit of RNA polymerase (De Angelis et al. 2007) were loaded.

Electrophoretic profiles were acquired by the Gel Doc System (Biorad) and digitally elaborated by the BioNumerics 4.5 software (Applied Maths). Fingerprints comparison and phylogenetic trees were by the UPGMA (unweighted pair-group method using arithmetic averages) method and the Pearson correlation coefficient (Rademaker and de Bruijn 2004) that, taking into account densitometry curves, is not affected by the typical drawbacks occurring when coefficients are determined by aligning gel bands (Boon et al. 2002; Krave et al. 2002).

8.2.7 Cloning of PCR Amplicons

PCR amplicons were cloned by the pGEM[®]-T Easy Vector Systems (II) kit (Promega) (Felske and Weller 2004). 5 U of T4 DNA ligase were used to ligate PCR products for 16 h at 4°C into pGEM-T vector at an insert:plasmid ratio of 6:1. The ligation product was added to 50 μ l of *E. coli* JM109 competent cells and the transformation was carried out by heating for 45 s at 42°C, cooling in ice, and incubating at 37°C for 90 min under shaking (750 rpm) in SOC growth medium (20 g Bacto[®]-tryptone, 5 g Bacto[®]-yeast extract, 10 ml 1 M NaCl, 10 ml 2 M Mg²⁺, 10 ml 2 M glucose, per liter).

100 μ l of each transformation was plated out on LB agar plates (10 g Bacto[®]-tryptone, 5 g Bacto[®]-yeast extract, 5 g NaCl per liter, 1.5% agar) containing ampicillin (100 μ g/ml), IPTG (isopropyl-BD-thiogalactopyranoside 0.1 M), X-GAL (5-bromo-4-chloro-3-indolyl-Betagalattoside, 50 mg/ml) (Sambrook et al. 1989), and incubated for 16–24 h at 37°C. The identification of CFU transformed by a PCR product was carried out by the white/blue selection method (Sambrook et al. 1989). The ligation strategy provides that if a nucleotide sequence is inserted into the plasmid, the lacZ operon is no longer expressed so that the synthetic chromogenic substrate is not processed, giving rise to white colored colonies instead of blue ones (positive clone) (Frackman and Kephart 1999). In addition, a colony PCR was also performed to evaluate the presence of 16S targeting amplicons into transformed cells (Frothingham et al. 1991). White colonies were picked up and resuspended in 50 μ l TE buffer (TRIS 10 mM, EDTA 1 mM, pH 7.5), incubated 15 min at 95°C to lyse cells. In order to make bacterial DNA available for PCR, 1 μ l of the latter suspension was added with 1 U Taq DNA polymerase (Eurotaq), 8 pmol each of the two primers T7–SP6 (Wang and Wang 1997; Zoetendal et al. 1998), 10 nmol each dNTPs, and 3 mM MgCl₂, to reach a final volume of 25 μ l.

The amplification conditions were the following:

95°C for 3 min (denaturing step)	} (25 cycles)
94°C for 10 s	
46°C for 20 s	
68°C for 60 s	
68°C for 10 min	} final elongation step)

PCR fragments were checked by electrophoresis on ethidium bromide (0.5 µg/ml) stained 1.5% agarose gel.

In order to avoid repeatedly sequencing of cloned fragments, a screening by amplified ribosomal DNA restriction analysis (ARDRA) was performed (Dunbar et al. 1999). Aliquots of the above described amplified fragments were digested by *Hinf*I, *Alu*I, *Rsa*I, and *Taq*I restriction enzymes (Promega) according to the manufacturer's conditions. The size of the digestion patterns was determined by electrophoresis on 2% agarose gel, in which adequate molecular weight markers were also run. PCR cloned amplicons, whose digestion profiles were different for at least one enzyme, were considered different and sequenced separately.

8.2.8 Sequencing and Phylogenetic Investigation

Plasmid DNA was extracted from the CFU that resulted positive to the insertion of 16S rDNA amplicons, as above described, by the QIAprep® Miniprep kit (QIAGEN, Inc., Chatsworth, California) according to the manufacturer's instructions and the inserted fragments sequenced by PRIMM.

Nucleotide sequences were compared with those available in the database "Ribosomal Database Project" (RDP 7.0) (Maidak et al. 1997), by the BLAST N system and the SIMILARITY_RANK (RDP) (Maidak et al. 1994). Sequence alignment was performed by the Bioedit and Clustal W programs (Thompson et al. 1994; Hall 1999). Sequence homologies were determined by the neighbor-joining method (Saitou and Nei 1987) and the Jukes and Cantor (1969) algorithm with "bootstrap" values calculated on a 1,000 replicates basis (Felsenstein 1985). Phylogenetic trees were built by the Treecon software (Van de Peer and De Wachter 1997).

8.3 Results and Discussion

In order to evaluate the structure of microbial communities in both bulk and rhizospheric soil, two molecular approaches were used (1) 16S rDNA PCR/DGGE, with an intermediate resolution capacity and (2) cloning/sequencing of 16S rRNA amplicons, that brings to a higher level of information. In particular, we investigated the short-term effects of soil management practices of the MESCOSAGR project,

including compost and catalyst treatments, on the diversity of soil bacterial communities.

Compost amendments affected the composition of bacterial communities which were tightly adhered to plant roots. These changes were even more evident when the whole community was investigated by total DNA amplification, instead of examining only the metabolic changes by following RNA extraction/amplification. It is likely that an enhanced nutrients availability due to the soil amendments may have quantitatively and qualitatively affected plant rhizodeposition that has in turn influenced the diversity and activity of the microorganisms colonizing the soil–root interface (Foereid and Yearsley 2004).

The TRA and MIN tillage practices in the Torino site did not appear to affect bacterial diversity, as it is shown by the gel and relative cluster analysis obtained for the rhizospheric soil DNA from the Torino experimental site after 1 year of treatment (Fig. 8.2). However, results of the COM treatment resulted to be different from other tillage practices. In fact, samples were pooled in two main groups: one containing all replicates from compost-amended plots (95.7% of similarity index), a second cluster in which almost all other samples had a 95.4% of similarity. This is also figuratively evident by DNA bands (one shown by an arrow) which are present only in compost-amended soils and indicate the changes occurred in the composition of bacterial communities induced by the compost additions.

Rhizospheric DNA, obtained from soils of the experimental site of Napoli, was similarly characterized by DGGE (Fig. 8.3) that shows that the changes in communities structure induced by compost amendments were evident after the second year of treatment, whereas TRA and MIN treatments did not have any

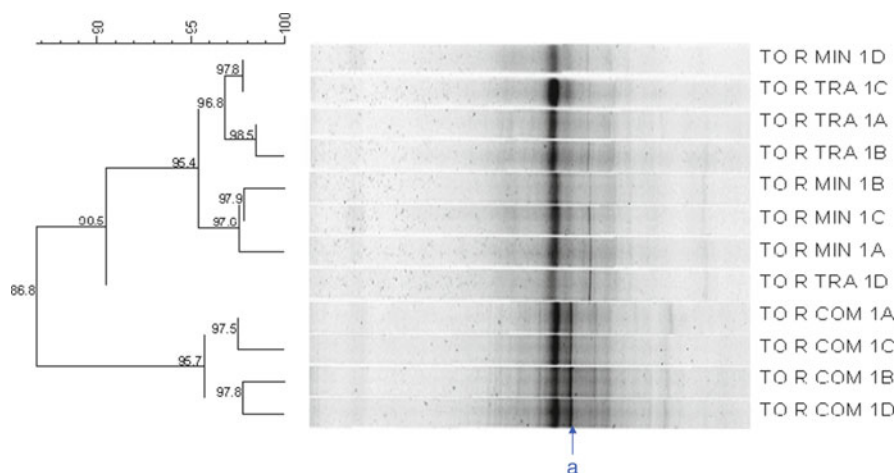


Fig. 8.2 Gel electrophoresis under denaturing conditions (DGGE) and relative cluster analysis. Rhizospheric DNA (R) extracted from soils under traditional (TRA) and minimal (MIN) tillage and compost (COM) amendment, after 1 and 2 years of treatments for the Torino (TO) site. Four replicates per treatment (A, B, C, D)

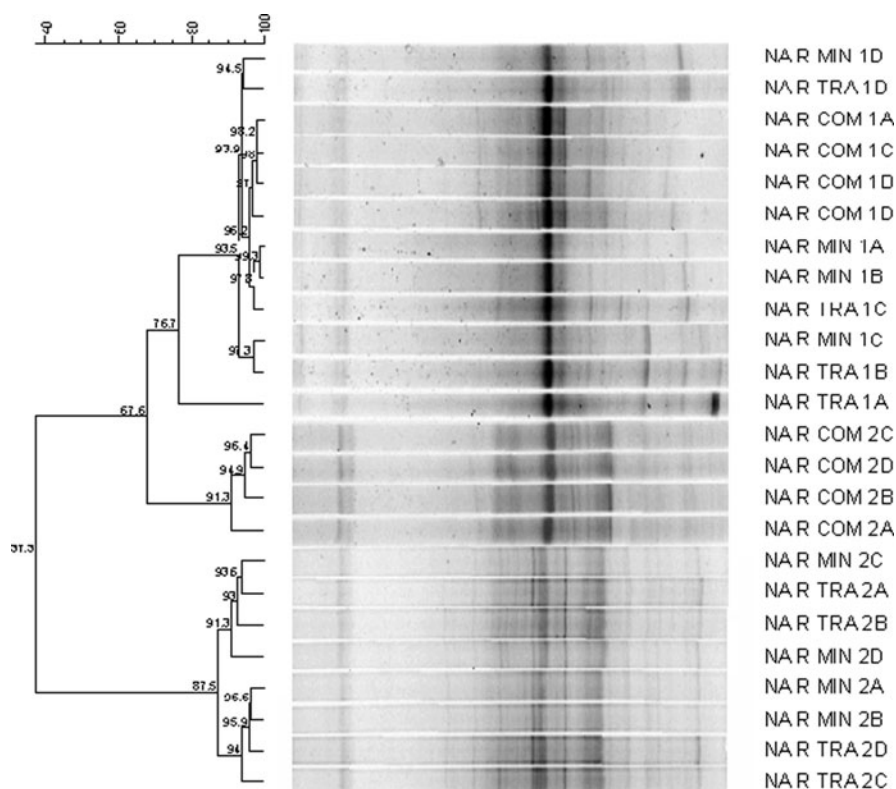


Fig. 8.3 Gel electrophoresis under denaturing conditions (DGGE) and relative cluster analysis. Rhizospheric DNA (R) extracted from soils under traditional (TRA) and minimal (MIN) tillage and compost (COM) amendment, after 1 and 2 years of treatments for the Napoli (NA) site. Four replicates per treatment (A, B, C, D)

effect on bacterial diversity. Cluster analysis also indicates that all samples extracted after the first treatment year were separate from those after the second experimentation year. This pool segregation may be attributed to climate and timing variability (annual temperature and rain, period of sampling). Since the analytical focus was on the bacteria colonizing the soil–root interface, the observed changes in diversity might be also due to different plant activities (growth, rhizodeposition, etc.).

Bulk soils from treated plots have been also investigated to evaluate the direct effect of applied treatments without taking into account the contribution of plant root exudates (data not shown). The community structure of soil bacteria were not significantly affected by either TRA and MIN tillage practices or compost amendments for the first year. Only a slight effect was noted after the second experimental year on both the whole communities and the metabolically active bacteria at time of sampling (after harvest).

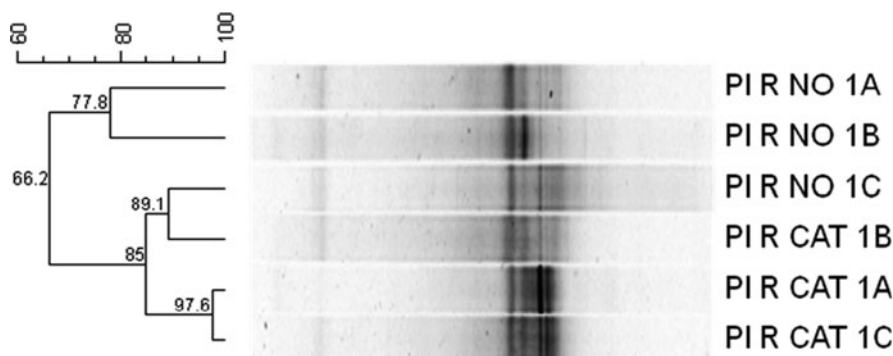


Fig. 8.4 Gel electrophoresis under denaturing conditions (DGGE) and relative cluster analysis. Rhizospheric RNA (R) extracted from soils amended with iron–porphyrin (CAT) and non-amended (NOCAT) after 1 year of treatment for the Piacenza (PI) site. Three replicates per treatment (A, B, C)

The addition of the iron–porphyrin biomimetic catalyst in the CAT treatment to stabilize SOM may have altered its mineralization rate, and thus, nutrients availability to microorganisms. We did not find any change in composition of bacteria communities for the soil in field plots of Torino and Napoli (data not shown). However, slight variations were noted for the experimental site of Piacenza, whose electrophoretic pattern showed that two out of three rhizospheric samples amended with the biomimetic catalyst (CAT) segregated separately from other ones (Fig. 8.4).

The effect of the catalyst on the microbial communities of the heavy textured soils from the Piacenza site may be attributed to the longer persistence of the iron–porphyrin on the surface of soil aggregates that for Napoli and Piacenza (see Chaps. 4 and 7). This effect is likely to be of more extent in following treatments years with additional amount of catalyst adsorbed on soil surfaces (see Chap. 3). However, it is noteworthy that the effect was shown in rhizospheric soil samples rather than in bulk soils, thereby suggesting that plant root exudates play a key role for life and diversity of soil microorganisms.

The cloning and sequencing approaches have been used to characterize bacterial populations at a phylogenetic level. In particular, following the DGGE characterization (Fig. 8.2), we also investigated the PCR amplicons relative to rhizospheric DNA for compost-amended (COM) soils (66 clones, marked with a C and progressively numbered) and TRA soils (62 clones, marked with an A and progressively numbered) of the Torino site after the first treatment year. After restriction analysis, that was necessary to avoid the sequencing of the same PCR fragment as described above, the selected clones sequenced were 52 and 41, respectively.

Our results indicate that 90% of sequenced clones showed a strict correlation with the Ribosomal Database Project sequences (Table 8.1). For all clones we report the classification in Table 8.1 only at the level of phylum, because in some cases the information were not enough to identify the level of species.

Table 8.1 Genomic library of clones amplified on rhizospheric DNA extracted from soil (a) amended with compost (COM) and (b) under traditional tillage (TRA) at the experimental site of Torino. Phylogenetic attribution and relative similarities values were by Ribosomal Database Project (RDP)

Clones	Phyla	%Identity
(a)		
C1	Proteobacteria	96%
C3	Acidobacteria	99%
C4	Proteobacteria	95%
C5	Actinobacteria	95%
C7	Proteobacteria	99%
C8	Acidobacteria	98%
C10	Uncultured	90%
C11	Uncultured	98%
C14	Acidobacteria	100%
C16	Proteobacteria	99%
C17	Verrucomicrobia	96%
C19	Actinobacteria	99%
C20	Proteobacteria	91%
C21	Actinobacteria	97%
C22	Verrucomicrobia	99%
C24	Proteobacteria	94%
C26	Acidobacteria	99%
C27	Acidobacteria	99%
C29	Proteobacteria	99%
C30	Acidobacteria	96%
C31	Actinobacteria	99%
C32	Acidobacteria	97%
C34	Uncultured	99%
C35	Actinobacteria	100%
C36	Proteobacteria	100%
C37	Actinobacteria	98%
C38	Proteobacteria	99%
C44	Actinobacteria	99%
C45	Firmicutes	99%
C47	Acidobacteria	98%
C48	Actinobacteria	99%
C49	Actinobacteria	97%
C50	Actinobacteria	96%
C52	Actinobacteria	98%
C53	Acidobacteria	100%
C54	Acidobacteria	99%
C56	Actinobacteria	96%
C57	Acidobacteria	98%
C58	Verrucomicrobia	97%
C59	Acidobacteria	99%
C60	Actinobacteria	99%
C62	Uncultured	99%

(continued)

Table 8.1 (continued)

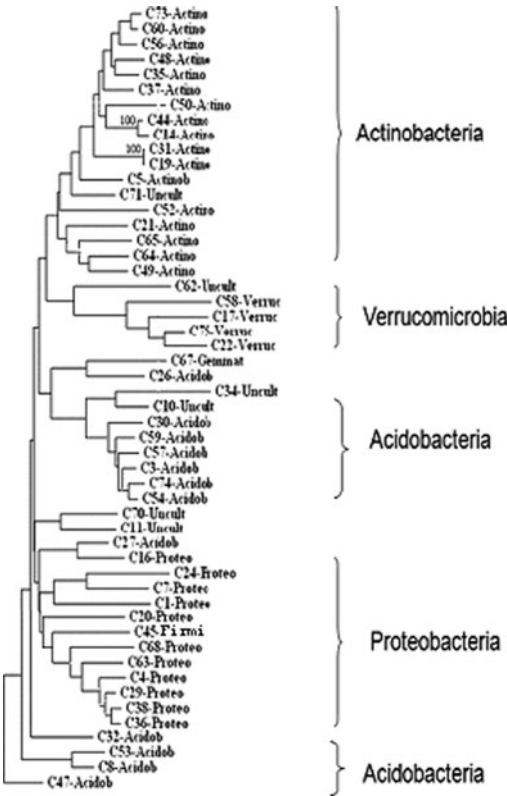
Clones	Phyla	%Identity
C63	Proteobacteria	97%
C64	Actinobacteria	97%
C65	Actinobacteria	99%
C67	Gemmatimonades	95%
C68	Proteobacteria	99%
C70	Uncultured	97%
C71	Uncultured	98%
C73	Actinobacteria	99%
C74	Acidobacteria	99%
C75	Verrucomicrobia	96%
(b)		
A3	Uncultured	85%
A4	Acidobacteria	99%
A5	Acidobacteria	96%
A7	Actinobacteria	98%
A8	Uncultured	93%
A9	Verrucomicrobia	98%
A12	Uncultured	98%
A22	Actinobacteria	97%
A23	Acidobacteria	98%
A24	Actinobacteria	96%
A26	Proteobacteria	99%
A27	Proteobacteria	100%
A30	Acidobacteria	96%
A33	Actinobacteria	96%
A36	Uncultured	98%
A37	Uncultured	100%
A39	Verrucomicrobia	98%
A40	Proteobacteria	99%
A42	Actinobacteria	100%
A44	Proteobacteria	96%
A46	Acidobacteria	99%
A71	Acidobacteria	97%
A91	Verrucomicrobia	91%
A93	Proteobacteria	97%
A94	Actinobacteria	99%
A96	Proteobacteria	93%
A97	Chloroflexy	96%
A100	Proteobacteria	99%
A105	Proteobacteria	88%
A106	Actinobacteria	99%
A107	Proteobacteria	95%
A109	Acidobacteria	93%
A112	Proteobacteria	98%
A115	Acidobacteria	100%
A122	Proteobacteria	100%

(continued)

Table 8.1 (continued)

Clones	Phyla	%Identity
A123	Actinobacteria	95%
A124	Acidobacteria	96%
A126	Acidobacteria	98%
A127	Proteobacteria	91%
A128	Uncultured	95%
A130	Verrucomicrobia	98%

Fig. 8.5 Cluster analysis and phylogenetic tree. Cloned and sequenced amplicons of rhizospheric DNA extracted from soil amended with compost (COM) for 1 year in the Torino site



The cluster analysis of the determined sequences (Figs. 8.5 and 8.6) shows the presence of four major groups corresponding to the *Proteobacteria*, *Acidobacteria*, *Actinobacteria* and *Verrucomicrobia* phyla. In particular, the library relative to the compost (COM) treatment indicates that *Proteobacteria* constituted about 23% (basically consisting of the γ -*Proteobacteria* subgroup), *Acidobacteria* 21.2%, *Actinobacteria* 34.6%, *Verrucomicrobia* 7.7%, *Firmicutes* 1.9%, *Gemmatimonadetes* 1.9%, and 9.7% of other bacteria of unknown phylogenetic classification (Table 8.2). In the library relative to soils under traditional tillage (TRA), *Proteobacteria* were 29.3% (mainly the γ -*Proteobacteria* subgroup), while *Acidobacteria* were 24.4%,

Fig. 8.6 Cluster analysis and phylogenetic tree. Cloned and sequenced amplicons of rhizospheric DNA extracted from soil under traditional tillage (TRA) for 1 year in the Torino site

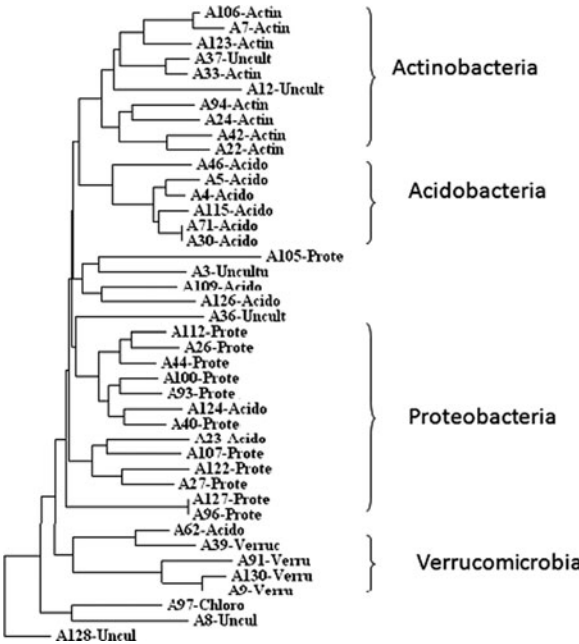


Table 8.2 Number of clones and their percentage (%) found in the two amplicons libraries for compost-amended (COM) soils (52 clones) and for traditionally tilled (TRA) soils (41 clones)

Phylum	COM	TRA
Acidobacteria	11 (21.2%)	10 (24.4%)
Actinobacteria	18 (34.6%)	8 (19.5%)
Chloroflexi	0	1 (2.4%)
Firmicutes	1 (1.9%)	0
Gemmatimonadetes	1 (1.9%)	0
Proteobacteria	12 (23%)	12 (29.3%)
Verrucomicrobia	4 (7.7%)	4 (9.8%)
Unclassified	5 (9.7%)	6 (14.6%)

Actinobacteria 19.5%, *Verrucomicrobia* 9.8%, *Chloroflexi* 2.4%, and 14.6% were bacteria of unknown phylogenetic classification.

In both libraries the prevalent group was that of the γ *Proteobacteria* subphylum, that is a bacterial group responsible for the mineralization of soil carbon and possesses the specific capacity for decomposition of fresh organic matter (Fontaine et al. 2003). Their abundance in the rhizosphere at the soil root interface is reasonable because plant rhizodeposition should account for an enhanced availability of microbial growth substrates.

The information provided by the two libraries suggests that the *Actinobacteria* is the only group that changes by a significant extent and increasing mostly in compost-amended soils. This variation is to be accounted for the capacity of

bacteria belonging to this phylum to degrade complex substrates such as the mature compost used in the amendments. The relative abundance of *Actinobacteria* in comparison to the total composition of the phyla in the whole sequenced communities agrees with the information provided by the DGGE profiles (Fig. 8.2). In fact, the profiles showed bands which were clearly present only in COM replicates and well indicated that compost-amended soils segregated separately from other soils.

Although the low number of sequenced clones reported here does not give a comprehensive view of the whole bacterial communities, our results do suggest that the application of different molecular approaches contribute to reach an advanced characterization of structure and diversity of soil bacteria and appraisal of their variation as a consequence of specific soil management practices.

Molecular approaches are undoubtedly a powerful tool to value microbial structure and diversity in different environments. Among different approaches, cloning and sequencing of whole metagenomes are still quite expensive and show some important drawbacks to be overcome. Very recently, pyrosequencing, a tool used firstly for the determination of the human genome, has been applied for soil metagenome investigation. It is based on the possibility to detect pyrophosphate released during DNA synthesis that is used to produce enzymatically ATP by ATP arylsulphatase; ATP is a suitable substrate for the luciferin–luciferase system that generates bioluminescence directly correlated to the amount of nucleotides incorporated during the PCR synthesis of template DNA (Ronaghi 2001). By running hundreds of thousands of sequences at the same time, each one detectable by a bar code system, and by the support of post-run softwares to manage huge amounts of data and to validate them statistically, it is possible to get much more details about microbial communities characterized by high diversities.

In conclusion, in the case of the MESCOSAGR project here reported, it appears that only the amendments with a mature compost had a significant effect on the soil microbial communities, while the addition of a biomimetic catalyst, used to increase C sequestration, did not have an effect as well as traditional and minimum tillage. By investigating and comparing bacterial communities inhabiting bulk and rhizosphere soil, it was also clear that the role played by plant root deposition; very likely root exudates make the nutrients brought by the compost amendment more available for bacteria, enhancing their diversity.

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Chapter 9

Measurements of CO₂ and N₂O Emissions in the Agricultural Field Experiments of the MESCOSAGR Project

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Abstract Soil organic matter (SOM) under agricultural management is a labile reservoir of C in the planet, and plays a key role in the production and emission to the atmosphere of two main greenhouse gases, CO₂ and N₂O. This chapter will overview one of the activities of the MESCOSAGR project that is the monitoring of CO₂ and N₂O emissions from soils under different agronomic treatments. The first part highlights the primary importance at global scale of SOM related to climatic change and agricultural management, including a description of processes involved in the CO₂ and N₂O evolution from soil. The state of the art of alternative techniques of SOM management in relation to CO₂ and N₂O emissions is discussed, also on the basis of the scarcity of literature data for Mediterranean croplands. The monitoring system of CO₂ and N₂O fluxes from field plots of the MESCOSAGR project indicated that compost additions were efficient in reducing fluxes from soils, especially for N₂O and for the large compost rates. The role of the compost humified organic matter in exerting a hydrophobic protection against SOM mineralization was thus supported. However, the soil treated with the biomimetic catalyst showed no difference in gases emission from control plots, thereby indicating that the fraction of SOM that was not photo-polymerized continued to be microbially mineralized together with the carbon rhizo-deposited by crop root systems.

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9.1 Sustainable Agriculture to Reduce Environmental Impact: Soil Organic Matter as a Source and Key Factor of Greenhouse Gases Production

Croplands play a key role for the sustainability of world's life. The exponential growth of global population and the need to produce alternative energy to fossil fuel has placed increased demands on agriculture to produce more food and biomass. For these reasons the agriculture poses a huge challenge for the sustainability at global and local environments, because of the different forms of environmental impacts related to agricultural management. The loss of soil organic matter (SOM) through enhanced mineralization and the emissions toward the atmosphere of greenhouse gases (GHGs), two processes strictly connected, are numbered among the environmental impacts of agriculture.

Net primary production (NPP) of the global croplands has been estimated at 15% of global terrestrial NPP (Field et al. 1998). Today, the increasing productivity is allowed by large use of fossil fuels and technologies, giving life to the so-called industrialized agro-ecosystems. These are wide extended, particularly in the developed countries. Cultivated lands cover 14% of the world's vegetated land surface while, across the European Union, agricultural surfaces cover an area of 46%, with 24% of arable and 19% of grasslands (Ramankutty and Foley 1999). The wide input of subsidiary fossil energy toward the agricultural systems improves the primary productivity, but mobilizing matter from other systems and speeding up the cycle matter inside the system, determines pollution and environmental degradation. In fact, one of the main effects of industrialized agro-ecosystem is the alteration of biogeochemical cycles, mainly carbon and nitrogen cycle. Particularly, the agricultural activities contribute considerably to the emissions toward the atmosphere of GHGs, i.e., carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O).

The main concern of the altered global C cycle is the large imbalance between carbon release to the atmosphere and carbon uptake by other compartments, that leads to a continued increase in atmospheric CO₂ to a rate of 4.1×10^9 tons of carbon per year (IPCC 2007a, b). CO₂ is considered the main GHG, affecting the phenomenon for more than 50% (IPCC 1996). The terrestrial carbon cycle is supposedly a sink of about 25% of the anthropogenic CO₂ emissions (Running 2008). On the other end, over the past few 100 years, the expansion of agricultural land has released substantial carbon to the atmosphere, due to soil carbon depletion by agriculture through removal of photosynthate carbon toward the market system and conventional tillage (CT) practices, which increase SOM mineralization rate (Schlesinger 1984).

Soil CO₂ emission is, after photosynthesis, the second largest C flux in terrestrial ecosystems, accounting for 60–90% of total respiration (Longdoz et al. 2000). Global soils, including crop soils, store great amounts of organic carbon (OC) and they are, potentially, one of the sinks of atmospheric CO₂ fixed by photosynthesis and released in soil as dead material and/or plant exudates. Global SOM has a basic role in carbon storage as well as in control of biological processes involved in soil–atmosphere

exchange of CO₂, CH₄ and N₂O. Despite its long residence times when in steady state, this very large C reservoir, which far exceeds C pools in both aboveground biomass and atmospheric CO₂ (Eswaran et al. 1993), represents a very large potential source of CO₂, if decomposition exceeds stabilization, i.e., humification.

Globally, plant biomass and SOM store about 500 Pg and 1,100 Pg C, respectively (IPCC 1996). Agricultural soils account for less than one-fourth of the SOC pool (Wood et al. 2000), and SOC levels are usually related to climate, topography and soil texture. Soils in North America, Asia and Europe are considerably richer in SOC (12.2, 12.6, and 14.6 kg C m⁻², respectively) than in sub-Saharan Africa (7.7 kg C m⁻²). However, the effects of human activity on global carbon stocks are inadequately understood, particularly for human-managed terrestrial ecosystems. One of the greatest uncertainties concerns changes in soil C stocks that may occur with different management.

Human activities have also dramatically altered the earth's nitrogen (N) cycle, strictly linked with the C cycle, particularly in the agro-ecosystems, since N immobilized in plant tissues is removed with harvest, thereby reducing the N availability in soil for the next crop cycle. Loss of N soil fertility is recovered by means of mineral fertilizers, whose production needs large amount of fossil fuels (Haber's process). Input of reactive N into the biosphere by man now exceeds the rate of biological N₂-fixation in native terrestrial ecosystems (Galloway et al. 2004). This increased reactive N is due not only by N fertilizer production, but also by the fossil fuel combustion used to support food and energy demands. The large input of added nitrogen and SOM management is responsible of a large diffusion of inorganic N in the environment with several impacts. A main impact is represented by the production of N₂O, this gas accounts for about 6% of the anthropogenic greenhouse effect (IPCC 1996), and agricultural soils are the major source of atmospheric N₂O. This gas is characterized by a warming potential 200 times as large as CO₂ and, by reacting with oxygen radicals in the stratosphere to form nitrogen monoxide, is involved in the destruction of stratospheric ozone (Crutzen 1981).

Thus, it is raising a new concern of industrialized agriculture toward a more environmentally sustainable system. Literature in sustainable agriculture identifies two core aspects (1) the over-exploitation of natural resources and (2) the induced pollution to the environment. Within this frame, agricultural soils play key roles, since they are natural resource with long regeneration cycle (centuries or millennia), and source of atmospheric degradation by emitted GHGs. In addition to developing new soil management strategies for sustainable agro-ecosystems, political and social approaches are also needed, based on a common understanding that soil and agro-ecosystems are essential for a sustainable society. For these reasons the sustainable management of soil received a strong support at the Rio Summit in 1992, as well as in Agenda 21 (UNCED 1992), the UN Framework Convention on Climate Change (UNFCCC 1992), Articles 3.3 and 3.4 of the Kyoto Protocol (UNFCCC 1998) and elsewhere. These conventions indicate the recognition by the world community of the strong link between soil degradation and desertification on one hand, and loss of biodiversity, threat to food security, increase in poverty, and risk of accelerated greenhouse effect and climate change,

on the other hand. Article 2 of the Kyoto Protocol states that nations adhering to the Protocol have to improve energy use in different sectors of the national economy, including agriculture, and promote policies for sustainable agriculture in order to reduce the impact on climate change. Moreover, article 10 invites nations to draft national or regional programmes to reduce GHGs emissions. In the same article, agriculture is considered as an economic sector liable to such planning. The conference of parties (COP) at Bonn and Marrakech (2001) have included evaluation of carbon sink in forest (article 3.3) and in agriculture (article 3.4), as a result of land use, land use change, and forestry (LULUCF). Improving the capacities of land use and agricultural practices to increase the carbon stocks in soils is one of the policies to be developed to promote SOM accumulation.

Following the results of the European Climate Change Programme (ECCP), a good progress is a policy that allows C sequestration from 5 to 8% of the CO₂ emitted by European activities. This topic is strictly associated to other problems of SOM management in southern part of Europe (Spain, Portugal, Italy, and Greece), where desertification is progressively advancing. SOM is often considered a key factor for either soil degradation or soil rehabilitation, and 2% has been suggested as the soil threshold content beyond which degradation occurs. According to UNEP (1991), desertification threatens over 60% of Southern European landscapes, and represents one of the largest environmental threats in the European Union.

SOM decline in cultivated soils has been studied in many long-term experiments (Grace and Oades 1994; Golchin et al. 1995). The restoration of organic matter in cropland is limited, while soil respiration (i.e., CO₂ release) tends to increase, thus resulting in a considerable carbon loss as compared with natural terrestrial ecosystems (Buyanovsky et al. 1987). Continuous cropping and inadequate replacement of nutrients, removed by harvested crops or lost through erosion, leaching, or gaseous emissions, deplete fertility and cause SOM levels to decline, often by 50% or more (Matson et al. 1997). When soils are tilled, SOM is more prone to weathering and decomposes fast because favourable conditions in water, aeration and temperature are available for microbial activity. The amount of organic matter lost by either clearing a wooded area or tilling native grassland varies according to soil type, but most of organic matter is lost within the first 10 years (Buyanovsky et al. 1987). Evidence acquired over several years increasingly indicate that certain fractions of SOC are likely to respond more rapidly than total soil C to land use change and management. SOM is divided into labile and non-labile materials. It has been shown that the C and N present in particulate organic matter (POM) can accumulate rapidly under land management systems that minimize soil disturbance and may also provide an early indicator of change in C dynamics and total soil C under different land use and management practices (Cambardella and Elliott 1992; Franzluebbers and Stuedemann 2002). The loss of SOM under cultivation can mainly be attributed to loss of the labile C fraction (Wadman and de Haan 1997).

There is a close relation between SOM degradation and global warming that, in turn, may strongly affect SOM decomposition. Rising temperatures brought about by climate change will cause microorganisms in world's soils to decompose organic matter more rapidly, releasing extra CO₂ and accelerating climate change

(Knorr et al. 2005). Over the short term, CO₂ increase in atmosphere may enhance plant growth through CO₂ fertilization, thus removing some of the excess CO₂ (Giardina and Ryan 2000). However, current models predict that, in longer term, rising temperatures will speed up decomposition of SOM and release CO₂ in atmosphere to an extent that exceeds any carbon sequestration in soil, and contributes further to climate change (Knorr et al. 2005). Some reports suggest that non-labile SOM is more sensitive to temperature than labile SOM, thus implying that the long-term effects of soil decomposition in a warming world may be even stronger than that predicted by global models (Davidson and Jansens 2006).

Despite the evidence that soil agricultural management is responsible of SOM degradation, the belief that agricultural ecosystems can play an important role in absorbing the surplus of carbon produced by human activities has been recently spread out. Such carbon sequestration would imply the transfer and secure storage of atmospheric CO₂ into persistent C pools, thereby preventing its immediate re-emission. Hence, a first step of this process is represented by an increased plant photosynthetic CO₂ fixation that is stimulated by the larger CO₂ concentration in atmosphere (Norby et al. 1992; Paustian et al. 1997). However, the photosynthate carbon should not be totally fixed into stable humus fractions, and variable amounts may be in large part distributed in rapidly cycling plant and soil carbon pools (Hungate et al. 1997; Schlesinger and Licher 2001).

Nevertheless, soil management strategies in croplands can have a great potential for carbon sequestration, since the carbon sink capacity for world's agricultural and degraded soil is still 50–66% of the historic carbon loss of 42–72 Pg (Lal 2004). However, the actual potential carbon storage in cultivated soils may be even smaller if climate change leads to increases in mineralization. Different literature showed that croplands have a great capacity to absorb carbon produced by fossil fuels burning (Schütz et al. 1990; Franzluebbers 2005; Johnson et al. 2005; Su 2007). Lal (2004) estimated that cultivated soil can accumulate 0.4–0.8 Pg C year⁻¹, if recommended farming practices are adopted, such as no-till, crop rotation, cover cropping and manure application. Lal (2001) reported that pasture treatment increased SOC from 9.2 to 55.4 Mg ha⁻¹ after 25 years, and forest management increased SOC from 14 to 48.4 Mg ha⁻¹ after 21 years. This suggests that grassland and pasture treatments would increase SOC stock as much as forest management. SOC sequestration might be increased at the expense of an increase of non-CO₂ GHGs emissions (CH₄ and N₂O), although soil-specific strategic practices, such as synchronized fertilization techniques and optimum water control, among other things, may reduce these emissions.

9.1.1 CO₂ Production from Soils

CO₂ from soil is not entirely produced by SOM degradation. In fact, vegetation contributes to total CO₂ emission with root and rhizo-microbial respiration. For this reason, the total soil CO₂ emission cannot be considered a direct measure of SOM

oxidation, despite some studies continue to interpret it in such a manner (Hanson et al. 2000; references therein). Since plants' contribution does not involve stable organic matter, total soil CO₂ flux has no effect on the long-term C balance in soils. Kuzyakov (2006) describes three C pools as sources of CO₂ from soil (1) SOM; (2) above and below ground dead plant residues; (3) organic substances released by living roots such as exudates, secretions and sloughed-off root cells. The last group is frequently described as rhizo-deposits.

The soil carbon pools are oxidized by different groups of heterotrophic organisms for their metabolic needs. The most important and active heterotrophs in soil are microorganisms: bacteria, fungi, actinomycetes and protozoans. The contribution of soil fauna (micro-meso and macro fauna) to total CO₂ emission from soils usually consists of only a few percent (Konate et al. 2003; Ke et al. 2005). Despite this negligible direct contribution, soil fauna may greatly affect microbial respiration by fragmentation of plant residues (Couteaux et al. 1991; Bonkowski et al. 2000) and by its different roles in the soil trophic webs (Bonkowski 2004). This intensifies their turnover rate and results in increasing CO₂ emissions from soil (Mikola and Setälä 1998; Wardle et al. 1998).

Contribution of plants' root respiration is most important and highly variable in space (soil characteristics, soil volume containing roots) and time (depending on environmental factors affecting plant activities). In particular, the contribution of vegetation to soil CO₂ fluxes is not constant and changes during the year. It depends on plant species (Fu et al. 2002), growth stage (Rochette et al. 1999), soil nutrients status (Bradley and Fyles 1996) and environmental factors, such as intensity of light for photosynthesis (Craine et al. 1999; Kuzyakov and Cheng 2001), soil moisture (Flanagan et al. 2002) and temperature (Buchmann 2000). Therefore, it is fundamental to distinguish the microbial decomposition of SOM in roots-free soil, frequently referred to as "basal respiration." Exudates, secretions and sloughed-off root cells from plants are liable to change microbial activity in the so-called "rhizosphere priming effect" (Kuzyakov 2002), and represent the interaction between growing roots and SOM decomposition (Cheng and Kuzyakov 2005). Similar changes in SOM decomposition have often been measured after addition of fresh plant residues to soil (Kuzyakov et al. 2000). The microbial capability in decomposing SOM depends on environmental factors (moisture, temperature, soil nutritional status, etc.) and SOM quality. Typically, SOM is divided into at least two and frequently more pools: inert (or passive), recalcitrant, resistant, decomposable, available, active, etc., each being characterized by different residence times. It is generally accepted that the inert pool is complex and tightly bound to clay minerals. This SOM pool has a very slow degradation rate with a mean residence time of thousands of years (Rethemeyer et al. 2004). This inert pool gives only a minor contribution to CO₂ fluxes from soil. The other SOM pools (decomposable, available, active) can be oxidized by microorganisms either through basal respiration or by priming effect.

With regard to the CO₂-driven greenhouse effect, only SOM-derived CO₂ contributes to change atmospheric CO₂ concentration. Due to fast turnover times, microbial decomposition of plant residues and rhizodeposits, as well as root

respiration, have no significant effect on C sequestration in the short- or long-term. Since plant C sources frequently amount to more than half of the total soil CO₂ flux (Hanson et al. 2000), the flux of plant-derived CO₂ masks the contribution of SOM-derived CO₂, when measuring CO₂ fluxes from planted soils.

9.1.2 N₂O Production and Emission from Soils

Soil N₂O production and emission is directly related to land use and soil management practices, since it is the biogenic product of microbial processes of denitrification and nitrification, as affected by physical–chemical characteristics of soil.

Bacterial denitrification is a respiratory reduction of nitrate and/or nitrite to gaseous NO, N₂O and N₂, coupled to phosphorylation electron transport. Many aerobic microorganisms use NO₃[−] as electron acceptor to derive energy from organic compounds when oxygen tension is low (heterotrophic denitrification), and, in this process, N₂O is an obligatory intermediate.

Microbial nitrification is the oxidation of ammonium to nitrite and nitrate, and, in most soils, autotrophic bacteria are responsible of this process, even though some studies suggest heterotrophic nitrifiers may also contribute to nitrification and N₂O production (Schimel et al. 1984; Anderson et al. 1993). N₂O is not an obligatory intermediate of nitrification process, since when O₂ supply is limited in soil (denitrification by nitrifiers), autotrophic bacteria can produce nitrous oxide by enzymatic reduction of nitrite. Autotrophic ammonium-oxidizing bacteria such as *Nitrosomonas europaea* can use NO₂[−] as an alternative electron acceptor under anaerobic conditions, thus reducing NO₂[−] to N₂O by the nitrite reductase enzyme (Firestone and Davidson 1989; Groffman 1991). The production of N₂O may also be caused by other microorganisms, e.g., during dissimilatory reduction of nitrate to ammonium, nitrate reduction to nitrite, and nitrate assimilation (Smith and Zimmerman 1981; Bleakley and Tiedje 1982). The mechanism of N₂O production by these bacteria and their role in N₂O production in soil require extensive study. Many fungi lack N₂O-reductase, so that N₂O results as the major product of fungal denitrification (Shoun et al. 1992). Laughlin and Stevens (2002) demonstrated that fungi were responsible for most of the N₂O production in a grassland soil. Spokas et al. (2006) also performed such inhibitor studies to elucidate potential mechanisms of N₂O production following chloropicrin fumigation in soil. Their results suggest that 20% of the N₂O production was from bacteria, while 70% of that was from fungi.

Typical key soil factors affecting the two microbial processes are: pH, temperature, total and mineral nitrogen content, labile organic matter availability, water content, soil aeration, redox potential. It is fundamental to distinguish between soil production and soil emission, since the N₂O produced by the two microbial processes does not necessarily leave the soil, depending on soil physical characteristics. Nitrification and denitrification can be active simultaneously in soil, since it is a complex and heterogeneous system with aerobic and anaerobic microsites, which are not homogeneously distributed, and, consequently, N₂O can

be evolved via both these processes (Nielsen et al. 1996; Abbasi and Adams 2000). At the same time, in microsites where anaerobic conditions are extreme, denitrification process can utilize N_2O produced by the same process or by nitrification, thus reducing the gas emission from soil.

Davidson (1991) presented a simplified model (hole in the pipe model) to describe the processes affecting production and emission of N_2O from soils. The model identifies three levels of control. Level I is represented by all factors affecting the rates of nitrification and denitrification; level II is for N_2O emissions depending on how soil physical–chemical parameters affect the ratio of end-products via both processes and on how fast; level III accounts for N_2O diffusing to atmosphere through the soil gaseous phase.

Among all factors, SOM plays an essential role. Soil labile organic matter is the reaction substrate for denitrifiers, thus favouring O_2 consumption and development of anaerobic microsites in soil, as well as enhancement of microbial activity. Many studies have found a significant positive correlation between N_2O fluxes and SOM content (Bremner and Blackmar 1981; Robertson and Tiedje 1984; Iqbal 1992), whereas mineral soils produce less N_2O (Duxbury et al. 1982). Several studies showed that in most mineral soil the key factor limiting denitrification is the availability of organic material, while others indicated that under soil anaerobic conditions, denitrifying activity is strongly regulated by the easily decomposable organic substances that are required to reduce nitrates (Burford and Bremner 1975; Paul and Beauchamp 1989; McCarty and Bremner 1992; Yeomans et al. 1992). Moreover, a reduction of the ratio $\text{N}_2\text{O}/\text{N}_2$ was observed with increase of easily degradable carbon materials in soil, since they appeared to promote a complete reduction of N_2O to N_2 (Elliot et al. 1990).

Typical soil managements, such as nitrogen fertilization and irrigation, are responsible for large N_2O fluxes. The great application of mineral-N fertilizers in traditional croplands, such as NO_3^- , NH_4^+ , NH_4NO_3 and NH_3 , is a key controller of microbial processes involved in N_2O evolution from soil (Bremner and Blackmar 1980; Duxbury et al. 1982; Dambreville et al. 2006). Irrigation is a fundamental crop management in arid lands characterized by cyclic water deficit, as in the Mediterranean regions. Several authors detected peaks of N_2O fluxes from crop soils following irrigation events, evidently as a result of enhanced denitrifying activities under restricted aeration state (Teira-Esmatges et al. 1998; Sánchez et al. 2001; Vallejo et al. 2004), while large emissions occur when irrigation is performed simultaneously or soon after N supply (Webster and Dowdell 1982; Ranucci et al. 2011).

9.1.3 Alternative SOM Management in Cultivated Lands

Different management practices can be listed for soil C sequestration in croplands, although their applicability might differ according to soil type and region (1) conservation tillage (zero or minimum tillage); (2) cover crops in rotation; (3) green

manure of cover crops; (4) cultivating crops with deep-root systems; (5) developing and cultivating plants with high lignin content, especially in residues and roots; (6) applying non-toxic exogenous organic matter (animal manure, compost). At present, few studies have been performed on the effect of this alternative soil management on soil CO₂ and N₂O fluxes.

Conservation tillage systems, including zero and minimum tillage, leave more surface residues because the soil is not turned over, thus creating less degradation and biological risk for soil erosion and, therefore, preserve SOM. Many studies on soil under long-term management involving CT and no-tillage (NT) practices have demonstrated that tillage causes a substantial decrease of SOM content and mineralization of carbon (Elliott et al. 1994; McCarty et al. 1995; Six et al. 1999). Johnson et al. (2005) summarized 44 studies regarding conservation tillage and showed that the rate of SOC storage in NT compared to CT has been significant, but variable, averaging $0.4 \pm 0.61 \text{ Mg C ha}^{-1} \text{ year}^{-1}$.

A counter-indication, often observed under no or minimum tillage practices, is the increase of surface residues with increased water retention, a major distribution of anaerobic microsites, and a reduced gas diffusivity that may contribute to enhance N₂O emissions (Situala et al. 2000; Forte et al. 2009). Robertson et al. (2000) measured an increase of 7.7% in soil N₂O emission, as compared with CT. An increased N₂O emission with NT represented a small offset (3.6%) of the SOC gain that occurred during 10 years of NT. Bremner and Blackmar (1980) observed increased soil N₂O fluxes shortly after mechanical disturbance by tillage and ascribed the phenomenon to release of N₂O-rich soil air. On the other hand, other works found larger denitrification rates and N₂O emission from undisturbed soil than for ploughed ones (Aulakh et al. 1984; Linn and Doran 1984; Staley et al. 1990), while Elmi et al. (2003) did not find any difference at all for denitrification and N₂O emission between no-tilled soil and soil cultivated by conventional and reduced tillage systems. The greater microbial activity involved in soil emission of N₂O might be dependent on the enhanced water-holding capacity of surface soil, as indicated by studies on no-till soils (Doran 1980). US research indicated that no-tilled soils had, on average, 1.4 times greater surface moisture than conventionally tilled soils. Other factors determining large fluxes, as the consequences of lack of soil disturbance in reduced tillage, are the reduction of macro pores (Lal 1997), increased soil aggregation (Doran 1980) and reduced aeration (Dowell et al. 1979).

The addition of exogenous organic materials (EOM) may be a tool to conserve organic matter and maintain or enhance soil fertility (Smith 2004), while also being effective in mitigating the rise of atmospheric carbon dioxide (CO₂) concentration (Follett 2001; Lal 2008). The management of organic residues has received much interest in recent years, as a means to increase the potential carbon sink of cultivated soils (Six et al. 1999) and improve control of nitrogen dynamics (Jenkinson 1985; Jarvis et al. 1989). The ultimate function of organic residues is to turn agricultural soil into sink for OC by enhancing the persistent pool of soil OC or the microbially stable humified matter, humic acids and humin in particular (Piccolo 1996).

Transformation of organic wastes (sewage sludge, green waste, industrial and organic waste, animal manure) into compost is becoming increasingly popular, thus

reducing the use of artificial fertilizers, and the amount of waste added to landfill sites. Compost is considered to be an environmentally safe, agronomically advantageous and relatively cheap organic amendment that stimulates soil microbial activity and crop growth (Garcia et al. 1994; Pascual et al. 1997; Van-Camp et al. 2004). Composting decreases the volumes of waste and their potentially dangerous organisms, becoming an important way to recycle organic matter from wastes. Composting or anaerobic digestion of animal manure and slurry together with straw, green wastes or other OM, in vulnerable areas, may also be useful to balance nutrients excess from nitrogen-rich areas to deficient areas. Soil amendment with organic N fertilizers has the advantage to recycle an already biologically fixed N, instead of industrially fixing additional N by the energy-intensive Haber's production of new mineral fertilizers. Hence, compost is a good supplier of N at low cost, considering that the average N content in the compost varies between 12 and 22 g kg⁻¹, in urban organic-waste compost and sewage sludge compost, respectively. More than 90% of total nitrogen content in compost is in organic form.

Treatments with different types of EOM (compost from green waste and from sewage sludge, biosolids) enhance organic matter and total N in soil (Ros et al. 2006; Mantovi et al. 2005; Zaman et al. 2004). According to Ayuso et al. (1996), this may be attributed to a direct effect of organic N in compost that is only slowly mineralized in soil (Castellanos and Pratt 1981). Ros et al. (2006) showed a significant increase of OC and ON in soils after 12 years of compost application, together with an increase in microbial activity, due to the combined effect of high-substrate C availability and direct microorganisms addition. Previous long-term field studies based on comparable organic amendments have shown similar effects. Zaman et al. (2004) found a noticeable effect on *Corg* in plots treated with sewage-sludge compost in a 37-year field experiment, while Canali et al. (2004) observed an increase in soil *Corg* content with dried poultry manure in a 6-year field experiment. In a 3-year field experiment, Madejon et al. (2003) reported a noticeable effect on *Corg* in plots treated with either organic waste compost or agricultural compost (olive mill wastewater mixed with other agricultural wastes). Ros et al. (2003) also observed an increase in *Corg* in a 2-year trial with organic waste compost. Moreover, organic fertilizer applications improve soil properties: aggregate stability and mineral nutrition of crops (Clark et al. 1998), pH stabilization, cationic exchange capacity (CEC) and water infiltration (Stamatiadis et al. 1999).

The effects of soil treatment with EOM, including compost, on CO₂ and N₂O fluxes from field soil are very complex, and the few existing experiments showed contrasting results. Studies conducted in field and by laboratory incubation, showed a general stimulation of microbial growth due to increased substrate-C availability, though a direct effect from compost-added microorganisms is also possible (Pascual et al. 1997; Garcia et al. 1998; Stamatiadis et al. 1999; Garcia-Gil et al. 2000; Ros et al. 2003). Basal respiration (CO₂ release) is considered a valuable indicator for C availability to sustain microbial activity (Insam et al. 1991). In a long-term study (12 years) aimed to compare effects of different composts, mineral fertilizers and compost plus mineral fertilizer (Ros et al. 2006), an increase of basal respiration and metabolic quotient ($qCO_2 = CO_2/C_{mic}$) was observed in soils that

had received compost. Adani et al. (2009) studied the effect of two rates (50 and 85 Mg ha⁻¹) of compost application to a soil cropped with maize. A stable soil respiration was found for compost-amended soils (CO₂ flux of 0.96 ± 0.11 g CO₂ m⁻² h⁻¹ and 1.07 ± 0.10 g CO₂ m⁻² h⁻¹, respectively, for 50 and 85 Mg ha⁻¹), whereas respiration increased in control with time.

Composted organic matter is rich in humic substances, and soil addition with water-extractable humic substances from compost was shown to enhance root uptake of nitrate and ammonium, through several mechanisms. Cacco et al. (2000) suggested that humic substances are directly involved in switching on nitrate transport genes in roots. Panuccio et al. (2001) suggested that water extractable humic substances specifically stimulated only NH₄⁺ uptake. Such improved nitrogen uptake by roots may lead to reduced mineral nitrogen transformation in N₂O, via denitrification and/or nitrification in anaerobic soil microsites. Conversely, it has been reported that organic fertilizers rich in readily available C compounds, such as volatile fatty acids (Paul and Beauchamp 1989) and hydrosoluble materials, stimulate soil biological N-processes such as nitrification (Müller et al. 2003) and denitrification (Loro et al. 1997; Rochette et al. 2000). These two microbial processes may easily induce N₂O production if anaerobic microsites are favoured in soils under minimum tillage and irrigation. Using a combination of organic residues with low and high C/N ratios, it may be possible to control N mineralization rates from organic amendments. Application of high C/N ratio organic residues may be a management tool to reduce N₂O emissions from amended soil. For example, Baggs et al. (2000) and Velthof et al. (2003) found that N₂O emissions from soil amended with organic materials of high C/N ratio were reduced due to N immobilization. Similar results were obtained by Ram et al. (2009), comparing low-nutrient green waste compost with feedlot manure rich in N. A similar study was performed by Meijide et al. (2007), who evaluated the influence of mineral and organic N fertilizers on nitrification and denitrification processes, and consequently on N₂O emissions. Their field experiment was carried out on an irrigated sandy loam soil under Mediterranean conditions during maize (*Zea mays* L.) growing season. The use of digested slurries mitigated N₂O emission by 25% in relation to untreated pig slurry. Denitrification appeared as the most important process responsible for N₂O emissions when organic fertilizers were applied to soil, while nitrification was most important in the case of inorganic fertilizer.

Manure application in modern cropping systems is known to sustain or increase SOC, while improving nutrient management and general soil quality. Manure addition may not be entirely beneficial, as increased CH₄ and N₂O emissions can occur.

A study, where composted pig manure and ammonium nitrate were compared for 7 years (Dambreville 2006), showed that 14 months after the last application pig compost increased potential denitrifying activity (+319%), N mineralization (+110%) and organication (+112%), whereas the N₂O/(N₂O + N₂) ratio resulted lower than the mineral fertilization. Fingerprints and analyses of clone libraries showed that the structure of the denitrifying community was affected by the fertilization type.

Proper management, such as avoiding excess manure application and synchronizing application time with crop uptake, will ensure the most positive effects of manure addition on SOC storage and GHG emission (Johnson et al. 2005). Soil N₂O emissions are enhanced by spreading animal manure as a slurry, since 60–70% of N in slurry is present as NH₄⁺, urea and uric acid, while solid manure and crop residues have larger content of less labile organic N materials. Moreover, slurry application of manure to soil surfaces can favour temporary anaerobic conditions leading to peaks in N₂O emissions (Vallejo et al. 2004; Mcswiney and Robertson 2005). In soils where the availability to microbial activity of labile organic material is limited, manure may produce more N₂O than mineral N fertilizers (Christensen 1983, 1985; Benkiser et al. 1987; Bowman 1990; Van Cleemput et al. 1992) and a combined application of manure and mineral fertilizers can lead to amplified N₂O emission rates.

9.2 Measurements of CO₂ and N₂O Fluxes from Soil

9.2.1 *State of the Art on Soil Gases Measurements*

Although agricultural soils are important source of anthropogenic CO₂ and N₂O, no alternative soil managements have been developed to limit their fluxes, particularly for N₂O. Since pedo-climatic conditions are key factors, a monitoring activity at territorial scale is needed, not only for testing different soil managements but also to obtain data from Mediterranean soil–crop systems. Freibauer (2003) has pointed out already that large uncertainties are present in the GHGs inventory for Mediterranean croplands due to lack of extensive monitoring activities.

Among alternative soil managements, minimum tillage, green and animal manure have been largely studied. Research on the use of compost in agricultural soils has been mostly focused on nutrition and environment aspects, i.e., OM quantity and quality, accessibility of organic contaminants and heavy metals, crops yield, soil microbial response. Fewer studies were devoted on GHGs emissions from soils following compost addition. Moreover, no examples are up-to-date present in literature for the use of catalysts in soils to structurally modify SOM and increase carbon fixation.

Monitoring GHGs fluxes from soil presents some difficulties. In the case of CO₂, it is difficult to discriminate among the different biogenic sources of CO₂ (see Sect. 9.1.2). The separation between SOM-derived and plant-derived CO₂ is essential to evaluate the real capacity of soil as source or sink of atmospheric CO₂. Also soil N₂O fluxes result from complex interaction among biological, physical and chemical factors, within a large spatial and temporal variability. Thus, the evaluation of soil fluxes of both gases is made difficult by methodological limitations (Kuziyakov 2006; Groffman et al. 2006), and high spatial and temporal variability in

field-scale, particularly for N₂O (Clemens et al. 1999; McSwiney and Robertson 2005; Wagner-Riddle and Thurtell 1998).

Models are increasingly used to quantify C and N gas fluxes at territorial scale, especially when agricultural policies are to be developed. However, there are few long-term data sets, particularly for N gas fluxes, to be used in model validation (see also Chap. 2). The current IPCC (2007a, b) methodology for producing national inventories of N₂O from agricultural land is based on the study of Bouwman (1994) and it assumes a default emission factor (EF) of 1.25% for soil-added nitrogen. This approach does not account for climate, management practices, irrigation, soils and crop types, and other variables. Moreover, the data considered by Bouwman (1994) were mainly referred to croplands under temperate climatic conditions. Thus, more experiments are required to obtain a correct evaluation of N₂O emissions from agricultural lands under different climatic regimes at regional and national scale.

Due to such shortcomings, new experimental designs for soil gases monitoring must be planned to obtain data with large time resolution. Spatial and temporal variabilities depend on the physical–chemical factors that affect all soil biological processes inducing the production of CO₂ and N₂O. Much of the challenge arises from the fact that small areas (hotspots) and brief periods (hot moments) often account for high fluxes. In the last decades several experiments were conducted to understand the factors controlling the CO₂ and N₂O fluxes from soils (oxygen content, nitrogen availability, soil moisture and texture, and so on). However, the complex regulation of these factors, including soil management practices, creates hotspots and hot moments that are difficult to quantify and model. Due to technical restrictions, most attention was focused in determining the hotspots, particularly for N₂O production and emissions. N₂O hotspots in soils involve the interaction among patches of organic matter and physical factors controlling oxygen diffusion in soil, and transport and residence time of N₂O in soil pores. Thus, a series of plant and soil factors, e.g., rooting patterns and soil structure at small (0.1–10 m) scales, topography, hydrologic flow paths and geology at larger (>1 km) scales, need to be considered to understand the spatial distribution of hotspots. Currently, soil N₂O emissions predicting models are calibrated on the basis of spatial variability. However, their reliability to predict temporal variations is seriously undermined due to the very few data available in literature to calibrate these models over time.

The hot moments concept has been known since long time but hardly investigated by continuous monitoring, particularly in the small time scale, since few experiments are based on high-time-resolution measurements systems [dynamic chambers, Tunable Diode Laser (TDL) associated with eddy covariance technique]. The large part of data produced up-to-now, are referred to manual chamber measurements limited in temporal resolution.

Despite the increasing popularity of the eddy covariance technique to assess ecosystem C exchange and, recently, also N exchange by means of TDL, classical static or dynamic chamber methods remain the most useful tools. This is due to some limitations of the eddy covariance technique for C exchange. Mainly, micrometeorological techniques are only able to obtain the total CO₂ fluxes and cannot partition total flux into its individual sources (Buchmann 2002). Conversely,

chamber methods allow CO₂ fluxes to be measured directly from soil. Moreover, the eddy covariance technique has large purchase and installation costs, particularly for TDL equipment, even though this has the additional advantage to provide soil exchange also for N₂O and CH₄ gases. Some studies have simultaneously used eddy covariance and chamber methods to separate net ecosystem CO₂ exchange from soil respiration (Lavigne et al. 1997; Dore et al. 2003), as well as to correct the fluxes obtained by eddy correlations during night periods (Anthoni et al. 1999; Law et al. 1999; Dore et al. 2003).

Factors affecting time variation of soil gas fluxes are different: management, root exudates, drying and rewetting, etc. Factors affecting time variation of soil gas fluxes are different: management, root exudates, drying and rewetting, etc. Contrary to current understanding that daily CO₂ dynamics are attributed to day–night variation of soil temperature, the process is also associated to fast decomposition processes that release easily decomposable substrates and enhance CO₂ production, thus resulting in diurnal CO₂ dynamics (Kuzyakov 2006). In agricultural ecosystems, pulse emissions of N₂O are also frequently associated with fertilizer additions, organic treatments and following re-wetting after periods of prolonged drought (Davidson et al. 1993; Ranucci et al. 2011).

9.2.2 Monitoring System in Field Plots

Within the MESCOSAGR project, soil CO₂ and N₂O fluxes were measured for each soil treatment in both the experimental sites of Torino (Tetto Frati) and Napoli (Torre Lama). Detailed information of study sites and experimental design are described elsewhere (Chaps. 3 and 4).

Two periods of gas-fluxes measurements (May 21–28 and July 16–24) were carried out in Torino (Tetto Frati) during the maize crop in 2008. The first period in May began immediately after nitrogen fertilization (where scheduled) and sowing; the second period was near the completion of maturity of maize plants. Gas emissions from the Napoli site were measured for all soil treatments during the autumn–winter period after the 2007 maize crop (October 2007–March 2008) and for the 2008 maize cropping season (May–August 2008). Conversely, the gas fluxes from soil plots treated with the water-soluble biomimetic catalyst (see Chaps. 3 and 4) and under wheat cropping were monitored in the period December 2007–August 2008.

Soil CO₂ and N₂O emissions were measured by means of an automated closed-chamber system coupled to a 1412-Photoacoustic Field Gas Monitor. The analytical system provided high-time resolution of gas fluxes data, being able to perform day-long analytical cycle of 10 min for each chamber. Before the measurements cycle in the Torino site and frequently (each month) in the Napoli site, tests to evaluate fluxes variability in space were performed. Since it was always low (coefficient of variation less than 100%), one or two chambers were placed in soil for each treatment. Each chamber provided daily, on average, 10–12 measurements.

Photoacoustic Field Gas Monitor operates collecting gas samples by means of pump from closed chamber; the gas sample is allocated in a small chamber (3 ml). Chamber is irradiated with pulsed, modulated by a chopper, narrow-band light. Gas absorbs light proportional to its concentration and converts it to heat. Temperature fluctuations determined by modulation generate pressure waves detected by sensitive microphones. Gas-specific carousel is available to select the appropriate light wavelength. The instrument is capable to measure gas concentration in few seconds.

However, the automated corrections for water interference and cross interference performed by photoacoustic system are unsatisfactory (Flechard et al. 2005). To counteract this limitation, the system was calibrated at different CO₂ concentrations and varying dew point temperatures. The data obtained by the photoacoustic system were compared with those produced by GC analyses. The experimental calibration calculated a correction factor for N₂O of +0.05 ppb for each ppm of CO₂.

Each chamber ($\emptyset = 30$ cm, $h = 10$ cm) is automated by means of electronic engine in order to modulate opening/closing cycle for the accumulation of soil air fluxes. Each cycle was run by a multiple channel sampler provided of ten channels. Each chamber was equipped with a vent valve to avoid pressure variations inside the chamber (Denmead 1979; Davidson et al. 2002; Bain et al. 2006). Inlet and outlet tubes allow air circulation from chamber to detection instrument.

Soil gases fluxes have been calculated for each chamber, considering a cycle of 3–5 measurements with open chamber and 10–12 measurements with closed chamber, covering a total time of about 10 min. The gas flux has been expressed as:

$$F_x = \frac{\Delta[x]}{\Delta t} \times \frac{V}{S},$$

where F_x was the soil flux of a specific gas x , $\Delta[x]$ was its variation of concentration expressed as mg/m³, in the time interval Δt , V was the chamber's volume and S the soil surface covered by it.

Linear regression was calculated for each measurement cycle, R^2 less than 0.8 has been rejected. The soil gases fluxes were calculated on time scale of 1 h after the following equation:

$$[x]_t = a(t - t_0),$$

where $[x]_t$ was gas concentration at time t while t_0 was the concentration measured for the first measurement performed with closed chamber. Multiplication of the angular coefficient a with S/V gave hourly fluxes.

Cumulative C and N fluxes from soils have been calculated. Gap filling has been obtained by the calculation of the mean fluxes of 3 days before the measurements interruption and the mean fluxes of 3 days of measurements resumption. The percent of emission has been also calculated as EF, that is the ratio of soil

cumulative N_2O fluxes over the amount of N applied to crop plots in either organic or mineral form.

The Napoli site has been also provided by a data-logger CR1000 (Campbell Scientific Ltd., Shepshed, UK) for continuous detection of soil temperature and moisture. The system was equipped of six probes for temperature detection (107 Temperature Probe Campbell Scientific) and three reflectometers for the detection of soil moisture (CS616 Water Content reflectometers – TDR, Campbell Scientific).

9.3 Summary Overview on CO_2 and N_2O Emissions from Field Plots

9.3.1 *Measuring Campaign of Maize Cropping in 2008 (Torino, Tetto Frati and Napoli, Torre Lama)*

Mean and cumulative values of soil CO_2 and N_2O fluxes, during maize crop in 2008, from different treatments of Torino site are shown in Figs. 9.1. Figure 9.2 describes the cumulative dynamics of CO_2 and N_2O soil fluxes from several treatments in the Napoli site for maize crop in 2008. Although the two sites were characterized by different soil characteristics and climatic conditions, duly recorded during monitoring campaigns, a common pattern for both gases has

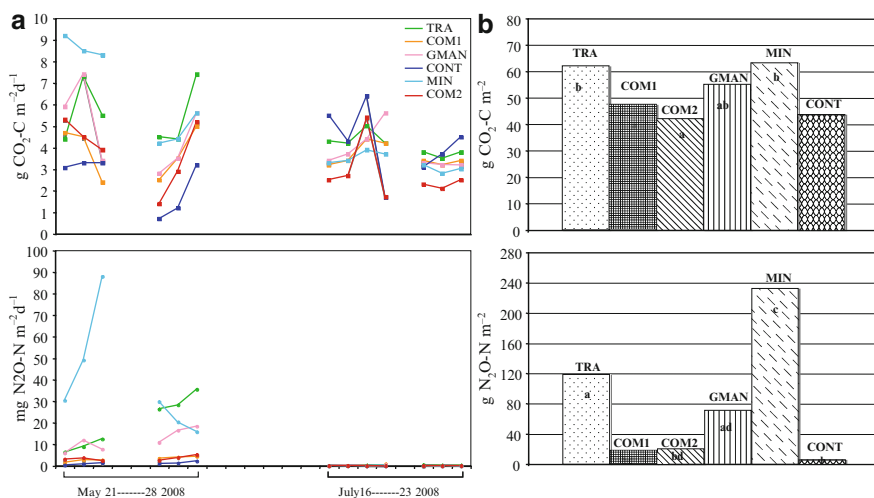


Fig. 9.1 Mean values (a) and cumulative dynamics (b) of CO_2 and N_2O fluxes from Torino (Tetto Frati) soil under maize crop in 2008. Different apical letters indicate significant differences among treatments (One-way analysis of variance + all pairwise multiple comparison procedures – Holm-Sidak method)

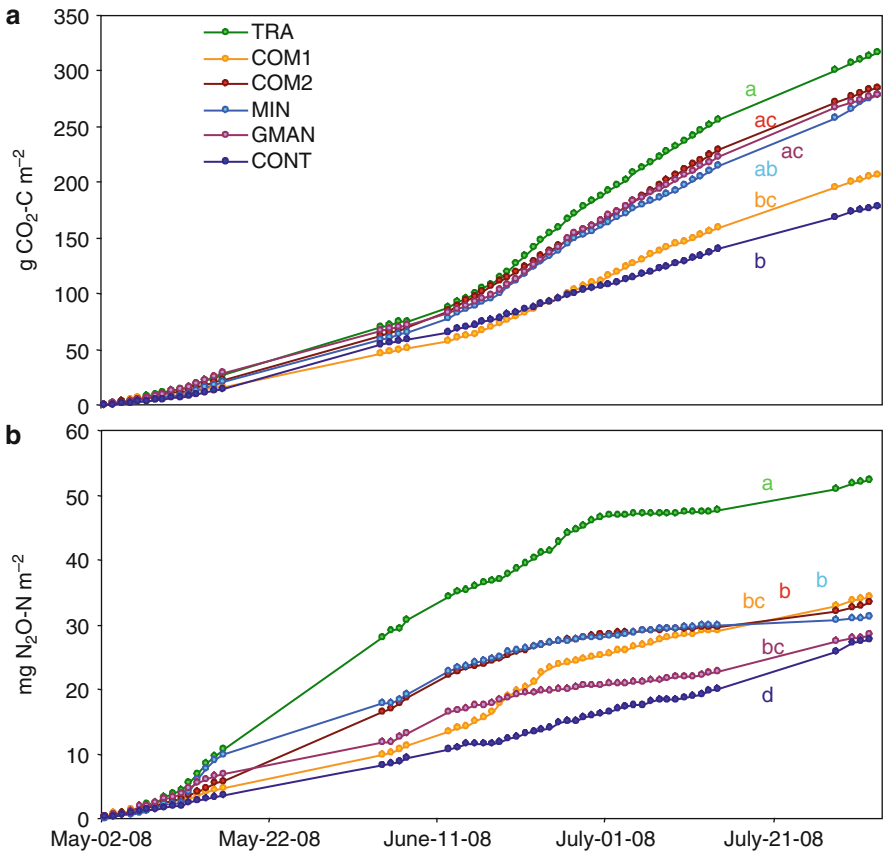


Fig. 9.2 Cumulative dynamics of CO₂ (a) and N₂O (b) fluxes from Napoli (Torre Lama) soil under maize crop in 2008. Different apical letters indicate significant differences among treatments (One-way analysis of variance + all pairwise multiple comparison procedures – Holm-Sidak method)

been observed. This suggests that, at least for a short time, soil management superimposes pedo-climatic factors.

The deep-plow traditional (TRA) treatment emitted more CO₂ from soil, as compared with other treatments in both sites, though statistically more significant against control (CONT) and compost (COM-1, COM-2) treatments. The reduced effect of the larger rate of compost (COM-2) on CO₂ emission was more evident in the loam soil of Torino, while the lower compost rate (COM-1) emitted less CO₂ in the clay soil of Napoli. As shown in Table 9.1, percent contributions on CO₂ emissions for each treatment, as compared to TRA and CONT, were similar in the two sites, with the exception of COM-2 in relation to CONT in the Napoli site. Soil C lost as CO₂ during maize cropping in TRA was 150 and 200% larger than for soil control treatment (CONT) in Torino and Napoli, respectively.

Table 9.1 Contribution of soil treatments on CO₂ and N₂O fluxes, as flux ratios of different treatments over either TRAditional or CONTrol treatment

	Torino maize in 2008	Napoli maize in 2008	Napoli maize in autumn–winter 2007–2008
CO₂ (g C m⁻²)			
COM-1/TRA	0.73	0.65	1.13
COM-1/CONT	1.1	1.31	3.94
COM-2/TRA	0.57	0.87	1.3
COM-2/CONT	0.85	1.75	4.55
MIN/TRA	0.87	0.86	0.7
MIN/CONT	1.3	1.72	2.44
GMAN/TRA	0.9	0.86	0.65
GMAN/CONT	1.35	1.72	2.28
TRA/CONT	1.5	2	3.5
N₂O (mg N m⁻²)			
COM-1/TRA	0.18	0.63	1.36
COM-1/CONT	1.5	1.32	4.07
COM-2/TRA	0.18	0.63	1.21
COM-2/CONT	1.5	1.32	3.62
MIN/TRA	1.16	0.58	0.97
MIN/CONT	9.7	1.2	2.91
GMAN/TRA	0.56	0.5	0.67
GMAN/CONT	4.7	1.04	2
TRA/CONT	8.3	2.08	3

The amount of CO₂ emitted by the two sites during maize crop in 2008 appeared essentially equivalent, ranging between 150 and 350 g C m⁻².

Soil N₂O emissions, monitored during maize crop in 2008, were greater of one order of magnitude in the loam soil of Torino than in the clay soil of Napoli (Figs. 9.1b and 9.2b). In both sites, CONT soils emitted significantly less N₂O than other treatments. Significantly larger emissions were observed in TRA and MIN treatments of Torino and in TRA of Napoli (Figs. 9.1b and 9.2b). In both sites, soil N₂O fluxes for the MIN, TRA and GMAN treatments appeared noticeable, particularly for the Torino site, corresponding to 970, 830 and 470% (Table 9.1), more than CONT, respectively.

Soil N₂O dynamics during maize crop in 2008 for all treatments and in both sites, showed large rates of emission in the first crop phase and typical end-point shapes (Figs. 9.1a and 9.2b), due to substrate (nitrogen) dependent process (see Sect. 9.1.2). N dependence of N₂O fluxes may be hypothesized to be strictly linked with crop phase and soil water content. However, no functional relationship was observed in both sites between N₂O fluxes and soil mineral N content, probably due to the interference by roots uptake. In fact, in the Napoli site, where a broad data set of N₂O fluxes and soil water content were produced during the 2008 maize cropping, a functional relationship was observed between fluxes and water-filled pore spaces (WFPS) only during the first stage of crop growth. At this stage, the

poorly developed root system of maize plants did not yet compete with nitrifiers and denitrifiers for mineral nitrogen (see Sect. 9.1.2). The soil water content monitored in the Napoli site and expressed as WFPS, ranged during the crop cycle between 60 and 90%, being this range widely described in literature as favourable for N₂O production (see Sect. 9.1.2). However, low N₂O emissions monitored in both sites during maize cropping may be also due to the N immobilization in the organic form, as proposed in Chap. 3.

9.3.2 *Measuring Campaign in the Napoli Site for the Autumn–Winter 2007–2008. Effect of Residues After the 2007 Maize Cropping*

Figure 9.3 shows the cumulative dynamics of soil CO₂ and N₂O emissions for all field treatments in the Napoli site after the 2007 maize cropping (October 2007–March 2008). Soil without management (CONT) constantly showed the lowest values of CO₂ and N₂O fluxes, as compared with other treatments. Soil CO₂ emitted from COM-2 was significantly larger than other treatments, including TRA. Both compost rates showed significantly greater N₂O fluxes than all treatments, probably due to slow mineralization of organic N from humified molecular associations, while organic N from GMAN was probably the fastest to be released. Minor contribution on soil N₂O fluxes of TRA and MIN during the autumn–winter period, and contrary to what observed during maize cropping, may depend on the fast depletion of mineral N added during the previous maize cropping season (see Sect. 9.1.3).

Moreover, ratio of fluxes from different treatments over those from TRA and CONT (Table 9.1) underlines the significantly lower CO₂ and N₂O emissions from the control soil. In particular, the CO₂ emitted from both compost rates, TRA and both GMAN and MIN was, respectively, about 400, 350 and 200% more than that released from CONT. A very similar trend was that observed for N₂O fluxes (Table 9.1).

9.3.3 *CO₂ and N₂O Emissions from Napoli Field Plots Treated with the Biomimetic Catalyst*

The cumulative dynamics of CO₂ and N₂O emissions from soils under wheat cropping of the Napoli site treated with (CAT) and without (NO-CAT) addition of the water-soluble biomimetic catalyst (see Chaps. 3 and 4 for additional details) are shown in Fig. 9.4. During the whole monitoring period (December 2007–August 2008), the emissions of both CO₂ and N₂O from the catalyst-treated

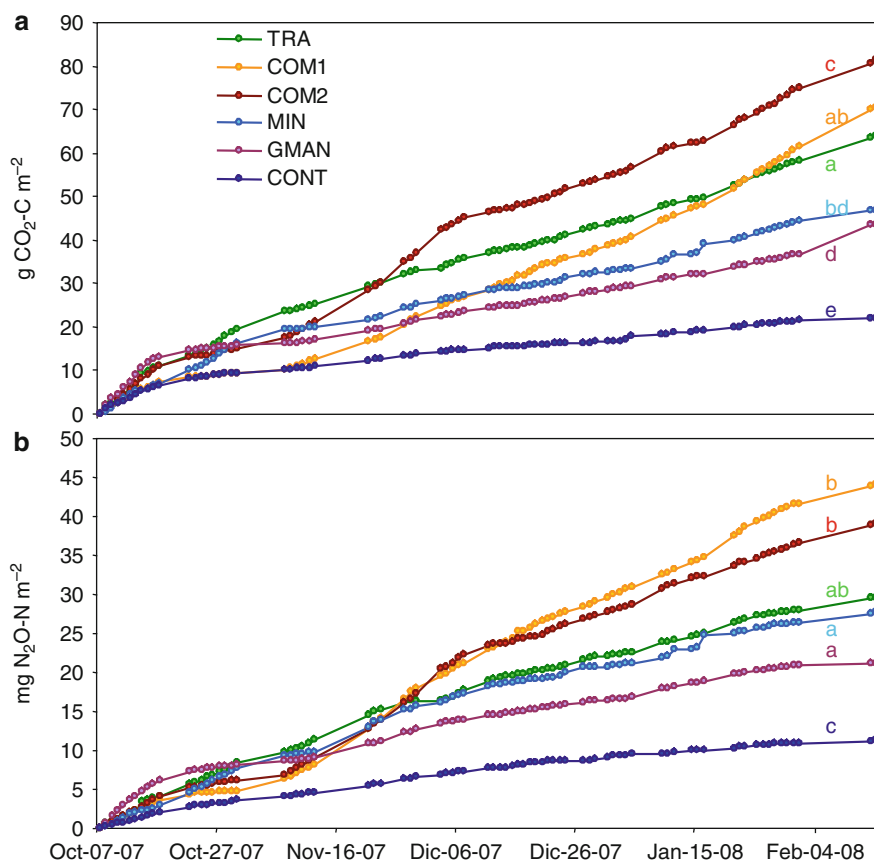


Fig. 9.3 Cumulative dynamics of CO₂ (a) and N₂O (b) fluxes from Napoli (Torre Lama) soil under maize crop during autumn–winter 2007. Different apical letters indicate significant differences among treatments (One-way analysis of variance + all pairwise multiple comparison procedures – Holm-Sidak method)

CAT soil resulted significantly larger than control (NO-CAT). Similar results were found for the CAT soil in the Torino site.

These results were unexpected, based on the assumption that the in situ photopolymerization of SOM would have stabilized the OC and inhibited soil respiration from CAT soils (see Chaps. 1 and 4). However, the actual experimental data, which were derived from fluxes directly measured in field plots, are in line with previous data and suggest a more complex effect of the catalyst-assisted in situ photo-oxidative reaction when in the presence of crop plants.

Piccolo et al. (2011) have found that different soils treated with the water-soluble iron–porphyrin did show both an improvement of soil structural stability and a significant decrease of soil respiration. They also showed that the effects of the

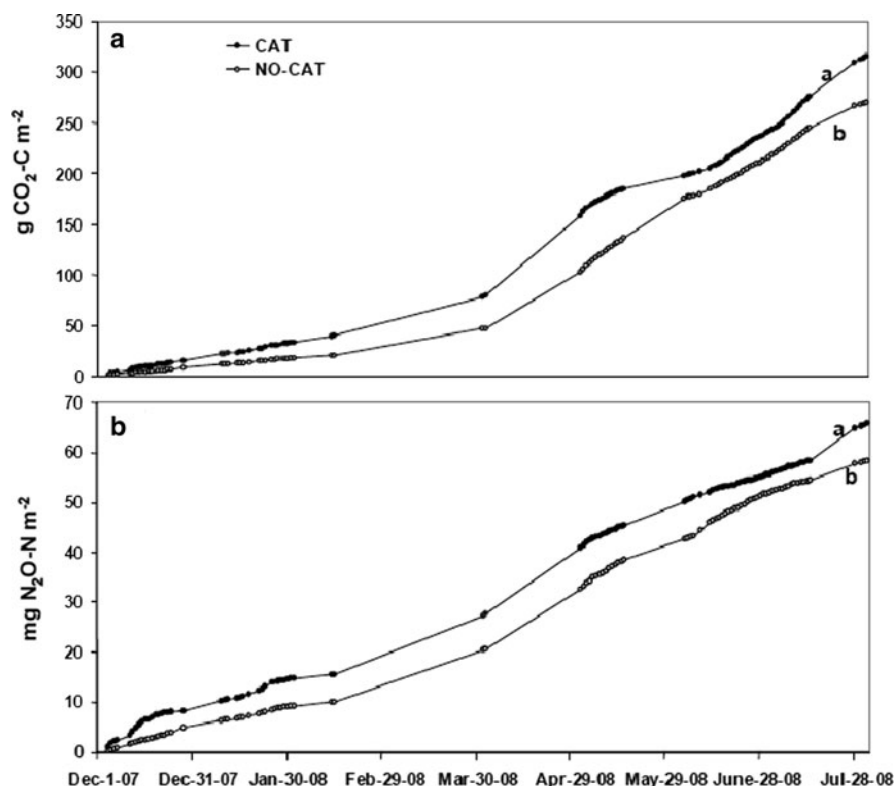


Fig. 9.4 Cumulative dynamics of CO₂ (a) and N₂O (b) fluxes from Napoli (Torre Lama) soil treated with (CAT) and without (No-CAT) catalyst under wheat crop. Different apical letters indicate significant differences among treatments (One-way analysis of variance + all pairwise multiple comparison procedures – Holm-Sidak method)

catalyst in the photo-oxidation of SOM were kept even after prolonged wetting and drying cycles. These results were confirmed by Gelsomino et al. (2010) by a microcosm experiment where they compared the effect of iron–porphyrin addition on either an unplanted soil or maize-planted soil. They found that soil CO₂ emission from the bare soil treated with catalyst was significantly reduced in comparison to control microcosm. Conversely, when the microcosm soil added with catalyst was cropped with maize, CO₂ emission was larger than control, and so was the maize root biomass.

Within the MESCOSAGR project, concomitant to such results of partial flux measurements from catalyst-treated field plots, a significant increase in carbon sequestration was found for the same soils after the three experimentation years in both Torino and Napoli sites (see Chap. 4). An explanation for these contrasting findings in two different measurements for the same soils (fixed carbon and respired carbon) may reside in the fact that the photo-polymerization of SOM under the

iron–porphyrin catalysis is effective only on phenolic humic molecules (Piccolo et al. 2011, Chap. 1). This signifies that these humic molecules are photo-oxidatively coupled into larger molecular-weight molecules and become less bio-available and, thus, persistent in soil, whereas all other aliphatic/alkyl molecules present in SOM (Nebbioso and Piccolo 2011) are still accessible to microbial mineralization. The implication is that such biolabile fraction of SOM, together with other labile molecules (proteins, saccharides, organic acids) rhizo-deposited by the crop root system, provide an organic C substrate for microbial degradation and ensure GHG fluxes that are not significantly different from the cropped control soil.

9.3.4 Soil N₂O Emission Factors (% EF)

The percentage of EF is an indication of the total amount of N lost as N₂O fluxes to the atmosphere. The EF calculated for each soil treatment in the Torino site during the 2008 maize cropping is shown in Fig. 9.5a, while the EF for soil treatments of the Napoli site is reported in Fig. 9.5b for the monitoring periods of autumn–winter 2007 and the 2008 maize cropping.

Very impressive is the amount of nitrogen lost to atmosphere during maize cropping in 2008 in the Torino site, particularly for the MIN, TRA and GMAN treatments (Fig. 9.5a). In the short period of 1 month, MIN and TRA lost about 6 and 5 kg of N ha⁻¹ year⁻¹, respectively, while GMAN lost more than 3 kg of N ha⁻¹ year⁻¹. For these three treatments the EF appeared significantly larger (MIN = 4.48%; TRA = 3.87%; GMAN = 2.2%) than the base value of 1.25% proposed by Bouwmann (1994) for added nitrogen. On the other hand, COM-1 and COM-2 emitted much less N during maize cropping in 2008, without significant difference between the two compost rates (Fig. 9.5a). However, EF of COM-2 was really one-half lower than COM-1, since it implied twice as much N as in COM-1 (Fig. 9.5a). All soil treatments in the Napoli site showed lower EF values than either the Torino site or the Bouwmann value, throughout maize cropping in 2008 and the overall period of monitoring (Fig. 9.5b). Also in the case of the Napoli site, the EF for the COM-2 treatment was the lowest of all treatments.

These values of nitrogen losses from field plots suggest that the large rate of compost addition was the most effective in sequestering N in soil, thereby confirming the mechanism of hydrophobic protection set up as one of the hypotheses of the MESCOSAGR project (see Chaps. 1 and 4).

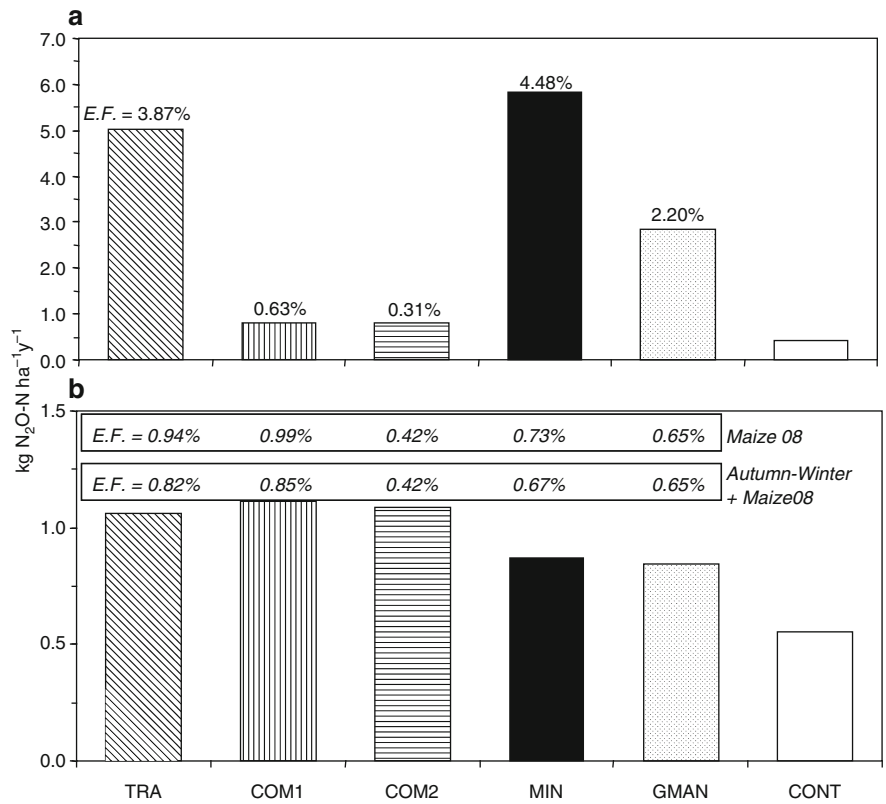


Fig. 9.5 Total N lost as N₂O from Torino (Tetto Frati) soil (a) during maize crop in 2008, and related Emission Factors for each soil treatments; total N lost as N₂O from Napoli (Torre Lama) soil (b) related to overall period of monitoring, Emission Factors for each treatment are reported for maize crop in 2008 and for autumn–winter 2007

9.4 General Remarks as Rationale for Future Experimentations

Our overall results highlighted some general remarks that are summarized as it follows:

1. On the basis of the long-term data set on field fluxes that was obtained for the Napoli site, we hypothesize a root effect on soil N₂O fluxes. It appears that the N dependence of N₂O fluxes could be strictly linked with crop phase and soil water content. During the early growth phase of the maize crop, where the root system was still poorly developed, nitrifiers and denitrifiers did not compete with roots for nitrogen supply.
2. Positive effects of compost addition on reduction of soil N₂O fluxes was observed in both sites, though the long-term data set of Napoli suggests that the phenomenon may be also dependent on crop phase. This may be due to either

a slow release of organic N from the humified compost material, or a greater N uptake by plants and its reduced availability to nitrifiers and denitrifiers. Moreover, an improved N uptake by roots may be facilitated by the bioactivity of humic molecules present in the soil solution. Residual effect of compost addition on N₂O emissions during autumn–winter period (after maize crop) is allowed by the slow mineralization of N, that becomes more available for nitrifiers and denitrifiers because maize root system no longer compete for N. Based on this, it may be interesting to also grow a winter cover crop under the same soil treatments, in order to prove a reduced effect of residual compost.

3. Our experimental data confirmed the large literature results reporting on the positive effect of reduced plowing in reducing soil N₂O and CO₂ emissions.
4. We once more observed that unmanaged control plots (i.e., soils in natural conditions) in different textured conditions (clayey soil of Napoli and loamy soil of Torino) are less metabolically active, and release less CO₂ and N₂O than managed soils.
5. Although our results did not show an effect of the catalyst treatment on the CO₂ and N₂O emissions from soil, fluxes measurements should be extended to longer time before attempting a definite conclusion on the effect of such a soil treatment.

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Chapter 10

Effects of Carbon Sequestration Methods on Soil Respiration and Root Systems in Microcosm Experiments and In Vitro Studies

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Maria Rosa Abenavoli, and Maurizio Badiani

Abstract In the framework of an interdisciplinary research devoted at increasing soil capacity to act as carbon sink by means of innovative and sustainable strategies (the MESCOSAGR Project), we studied, in microcosm-scale model systems, changes of selected soil chemical properties, soil CO₂ efflux, and root morphology after addition of either mature compost or a biomimetic catalyst (CAT) (synthetic water-soluble iron–porphyrin), as single addition or in combination of the two treatments. Direct effects of CAT on seed germination, seedling establishment, and plant growth were also evaluated in model plant species. When applied to bare soil, CAT was able to reduce CO₂ emission from soil. Soil amendment of compost alone stimulated CO₂ emission from soil, whereas its combined addition with CAT strongly depressed the compost-induced CO₂ release. In planted microcosms, the contribution of the rhizosphere-derived CO₂ efflux markedly increased the total soil respiration and CAT addition further stimulated CO₂ release from soil. It is thus suggested that iron–porphyrin, growth of maize root, and CO₂ release are functionally interconnected. The increased total soil respiration observed in planted systems may be due to a larger contribution of the rhizosphere-derived CO₂ efflux, as a consequence of secondary actions or specific mutual interactions of the catalyst-root system. The direct CAT effect on model plant species implied a complex pattern of dose-dependent, and, remarkably, species-specific responses, as observed in both root systems and aerial plant parts. The observed strong CAT promotion of the synthesis of photosynthetic pigments might indicate an in planta uptake and translocation of the CAT molecule, prompting to envisage potential applications of this molecule in a wider agro-biotechnological context.

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Abbreviations

CAT	[<i>meso</i> -tetra(2,6-dichloro-3-sulfonatophenyl) porphyrinate of Fe(III) chloride]
CEC	Cation exchange capacity
Chl	Chlorophyll
Chlide	Chlorophyllide
EC	Electrical conductivity
F	Root fineness
LR	Root length
MS	Murashige and Skoog medium
Pchlide	Protochlorophyllide
POR	NADPH:protochlorophyllide oxidoreductase
RMR	Root mass ratio
SOM	Soil organic matter
TD	Tissue mass density
TI	Topological index
TN	Total nitrogen
TOC	Total organic carbon
VR	Root volume
WP	Plant dry weight
WR	Root dry weight
WS	Shoot dry weight

10.1 Introduction

Enhancing CO₂ sequestration in soil – that is, the transfer of atmospheric CO₂ into a persistent terrestrial pool – can be considered an efficient strategy for balancing the atmospheric CO₂ enrichment due to anthropogenic emissions, which is one of the main factors contributing to global climate change (Lal 2007).

Recent new insights into the soil organic carbon cycle suggest that some sustainable management practices, such as (1) protecting easily biodegradable organic fraction by means of humified and hydrophobic organic compounds, such as compost from agricultural by-products (Piccolo and Teshale 1998), and (2) oxidative polymerization of soil organic matter (SOM) by catalysis, or biomimetic photosensitization in the presence of solar radiation (Piccolo et al. 2011), appears to be particularly promising to increase carbon sequestration by agricultural soils and reduce greenhouse gas emissions.

In such context, Piccolo et al. (2005) and Šmejkalová and Piccolo (2005) have been recently proposing the use of a synthetic, water-soluble iron–porphyrin [*meso*-tetra(2,6-dichloro-3-sulfonatophenyl) porphyrinate of Fe(III)chloride] as a chemical catalyst (CAT, in the following) capable to mimic the action of soil microbial enzymes involved in the oxidative polymerization of natural precursor of humic molecules (see Chap. 1).

The MESCOSAGR Project has been an interdisciplinary effort specifically devoted to increase the carbon sink capacity of soils by means of innovative and sustainable strategies, as they affect physical, chemical, biological, and agronomic soil quality. As a contribution to the MESCOSAGR Project, we studied, in soil microcosm experiments, the changes of selected soil chemical properties, soil CO₂ efflux, and root morpho-topology after addition of either mature compost or a synthetic, water-soluble iron–porphyrin biomimetic catalyst (CAT), as single addition or in combination of the two treatments.

Enclosed model ecosystems, such as microcosms, have become a major research tool in soil ecology to enable scientific investigations in less complex ecosystems than in natural ones (Kampichler et al. 2001). Soil microcosms are nowadays increasingly being used to also investigate changes in soil CO₂ emission in differing soil–plant systems under differing treatments (Smart and Peñuelas 2005). Even though they suffer from disadvantages and limitations (i.e., distance from reality and scale problems), soil microcosms allow a large number of replicates in short-time periods and at reasonable costs. Moreover, they are useful for developing hypotheses about soil–plant relationships, before being further tested at the appropriate scale in field experiments (Lukkari et al. 2006). In a comparative study, Tingey et al. (2008) recently provided evidence that results from enclosed ecosystems can be extrapolated to field conditions. In fact, they found that enclosed model ecosystems displayed similar patterns as to field experiments, when measuring photosynthesis and soil respiration in a soil–litter–plant system. Finally, enclosed model ecosystems such as mesocosms or microcosms units have been efficiently used in recent studies to investigate changes in soil respiration, as induced by compost amendment, nature and properties of the growth medium, or CAT additions (Gelsomino et al. 2010; Tortorella and Gelsomino 2011).

Furthermore, direct CAT effects on seed germination, seedling establishment, and plant growth were evaluated during the early growth phases of seedlings belonging to model plant species, namely garden cress (*Lepidium sativum* L.), carrot (*Daucus carota* L.), and *Arabidopsis thaliana* (L.) Heynh, grown under sterile conditions. Particular attention was paid to the effects on roots, because of their well-known ability to modulate quickly their own morphology and development in response to changing conditions in soil, as well as in response to a multitude of external stimuli.

10.2 Materials and Methods

10.2.1 Microcosm-Style Experiments

10.2.1.1 Experimental Setup

The microcosm-scale experiment was carried out in a greenhouse facility of the Mediterranean University of Reggio Calabria. The microcosms consisted of 9.5 L PVC pipes (30 cm height × 20 cm diameter) filled with approximately 13 kg of a

soil/perlite (80/20, v/v) mixture. The soil was a clay loam [sand 36.0%, silt 32.0%, clay 32.0%; bulk density $1.23 \pm 0.04 \text{ kg dm}^{-3}$; pH-H₂O 7.2 ± 0.2 ; pH_{KCl} 6.4 ± 0.1 ; total organic C (TOC) $19.3 \pm 0.4 \text{ g (kg dw [dry weight basis] soil)}^{-1}$; total N (TN) $1.8 \pm 0.2 \text{ g kg}^{-1}$; C/N ratio 10.7; NH₄⁺-N $17.1 \pm 1.0 \text{ mg kg}^{-1}$; NO₃⁻-N $13.0 \pm 1.0 \text{ mg kg}^{-1}$; Olsen P $18.3 \pm 2.3 \text{ mg kg}^{-1}$; total CaCO₃ $8.4 \pm 1.0 \text{ g kg}^{-1}$; active CaCO₃ $3.9 \pm 0.2 \text{ g kg}^{-1}$; cation exchange capacity (CEC) $17.1 \pm 1.7 \text{ cmol}_{(+) } \text{ kg}^{-1}$; electrical conductivity (EC at 25°C) $0.165 \pm 0.004 \text{ dS m}^{-1}$ ($1 \text{ dS m}^{-1} = 1,000 \mu\text{S cm}^{-1}$)] sampled from the surface (0–15 cm) Ap horizon of an agricultural field located in the Agricultural Experimental Station of the Mediterranean University of Reggio Calabria. After coarse sieving (4 mm mesh), the freshly collected soil was thoroughly mixed with commercial perlite (Agrilit[®]3, purchased from Perlite Italiana s.r.l., Milano, I) at 70/30 (v/v) soil/perlite ratio. Perlite properties were: particle size 2–5 mm, bulk density $100 \pm 20 \text{ kg m}^{-3}$, pH 6.5–7.5, CEC $0.79 \text{ cmol}_{(+) } \text{ kg}^{-1}$, EC 0.020 dS m^{-1} , water content at –1 kPa 26.30%, water availability 12.97%. Microcosms were closed at the bottom by a thin layer of nylon stocking material, separated from the soil/perlite mixture by a 1-cm drainage layer of water-washed quartz sand. Compost from agricultural by-products (pH-H₂O 8.7, EC_{1:10} 3.357 dS m^{-1} , TOC 28.75%, TN 2.24%, C:N ratio 12.8) was provided by GESENU (Perugia, Italy). The water-soluble iron–porphyrin [*meso*-tetra(2,6-dichloro-3-sulfonatophenyl) porphyrinate of Fe (III)chloride], here referred to as the biomimetic catalyst (CAT), was synthesized according to the procedure of Traylor et al. (1984) as modified by Piccolo et al. (2005).

The microcosms were randomly set up in a 2×4 experimental block (6 times replicated) with two plant treatments (with and without maize plants) and four soil treatments, namely:

- Soil/perlite mixture without compost or synthetic iron–porphyrin, as control treatment (CONT).
- Soil/perlite mixture amended with compost at 2 kg m^{-2} rate (COM).
- Soil/perlite mixture added with 1 g m^{-2} synthetic iron–porphyrin (CAT); by assuming a porosity of 50% and a 15% volumetric water content at field capacity, the above CAT rate would have yielded an approximate concentration of $10 \mu\text{M}$ CAT in the soil solution.
- Soil/perlite mixture amended with compost (2 kg m^{-2}) upon microcosms filling, and then surface sprayed (see below) with iron–porphyrin (1 g m^{-2}) (COM + CAT) 10 days after microcosms filling.

The synthetic iron–porphyrin was surface sprayed upon CAT and COM + CAT treatments as a buffered solution (100 mM phosphate buffer, pH 7.0) soon after microcosm filling. De-ionized water was periodically added to soil microcosms to maintain the moisture between 13 and 15% of total soil volume.

Pre-germinated maize seeds (*Zea mays* L., var. *Cecilia*, kindly provided by Pioneer HI-Bred Italia s.r.l., Parma, Italy) were transplanted into soil microcosms 55 days after the beginning of the trial (one seedling per microcosm) and left growing for additional 21 days.

During the 76-day experimental period, greenhouse air temperature fluctuated between 12 and 25°C according to the day/night cycle and depending on external weather conditions, while soil temperature varied slightly within a range of 16–21°C.

10.2.1.2 Plants Sampling and Root Morpho-Topological Analysis

At the end of experimental period, soil microcosms were destructively sampled and 21-days-old maize plants were gently separated into shoot and root, rinsed with de-ionized water, and their fresh weight was determined gravimetrically. Shoot dry weight (WS, g) was measured after drying in an oven at 70°C for 48 h. The root system was stained with 0.1% (w/v) toluidine blue O for 5 min and then scanned at a 600 dpi resolution (WinRhizo STD 1600, Instruments Régent Inc., Canada).

For topological analysis (Fitter 1986), the magnitude (μ), the number of external links in the root system, and altitude (a), the number of links in the longest single path, were measured with WinRhizo Pro v. 4.0 software package (Instruments Régent Inc., Canada). These parameters allowed to calculate the topological index ($TI = [(\log a)/(\log \mu)]$) (Glimskär 2000). TI values close to 1 indicate a “herringbone” root structure, where branching is largely confined to a main axis, whereas values close to 0.5 indicate a dichotomous root structure characterized by more random branching.

For morphological analysis, the length (LR, cm) and the volume (VR, cm³) of the whole root system and the total root length within diameter classes (“fine roots”: 0–0.4, 0.4–0.8, and 0.8–1.2 mm; “coarse roots”: 1.2–1.6, 1.6–2.0, 2.0–2.4, 2.4–2.8, and >2.8 mm) were measured (WinRhizo software). Subsequently, root dry weight (WR, g) was determined after oven-drying (70°C) until a constant weight was reached. Total plant dry weight (WP, g) was obtained by summing WR and WS. Based on the measurements above, root fineness [$F = LR/VR$], cm root length (cm³ root volume)^{−1}] and tissue mass density [$TD = WR/VR$], g root (cm³ root volume)^{−1}], which represent structural root parameters, were calculated. These parameters are linked by the following relationship: $LR = WR \cdot (F/TD)$ (Ryser and Lambers 1995). Furthermore, root mass ratio [$RMR = WR/WP$], g root (g plant)^{−1}] was determined to evaluate the relative biomass allocated to roots.

10.2.1.3 Soil Analysis

Soil respiration was periodically monitored by using a closed dynamic soil CO₂-flux system (LI-8100 automated soil CO₂ flux system, LI-COR Inc., Lincoln, Nebraska, USA) equipped with a 10-cm survey chamber for measuring soil CO₂ efflux ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Volumetric soil moisture (ECH₂O EC-5 probe, Decagon Devices, Pullman, WA, USA) and soil temperature (Omega probe, Type E, LI-COR Inc.) were also monitored (at 5-cm depth) over time. Soil samples were

collected at the beginning (day 0) and at the end of the experimental period (day 76). The pH, EC, TOC, and TN were determined using standard methods (Sparks 1996).

10.2.1.4 Statistics

Soil variables, plant growth, and root morphological and topological parameters were firstly checked for deviations from normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene median test). Since the block effect was not significant ($p > 0.05$), the data were subjected to statistical analysis by using the Systat 12.0 software (Jandel Scientific, San Rafael, CA, USA). Tukey's post hoc test comparison (at $p < 0.05$) was applied for pairwise comparison of means. Data of Figs. 10.10 and 10.11 were statistically processed by two-way analysis of variance (ANOVA, CAT $\times$ COM) with repeated measures and three-way ANOVA, CAT $\times$ COM $\times$ plant with repeated measures, respectively, in order to give prominence to the main effect of the treatments (CAT, COM, and maize plant) alongside the variability given over time.

10.2.2 Laboratory Studies on Seed Germination and Post-germinative Processes

Direct *in planta* effects of CAT on seed germination and seedling establishment were evaluated on two fast growing and easily cultivable species amply used for laboratory studies, such as garden cress (*Lepidium sativum* L.) and carrot (*Daucus carota* L.). The used CAT concentrations were meant to be in the same range of those used for the microcosm experiments (see above); 0 (control), 3, 9, 27, 81, or 243 μM . These were obtained by diluting, in sterilized 10 mM phosphate buffer pH 6.0, the appropriate volumes taken from a sterilized stock of a 10 mM CAT solution in the same buffer. Previously sterilized seeds were germinated at 20°C in the dark in Petri dishes containing filter paper disks imbibed with CAT solutions at the concentrations stated above. Radicle protrusion was assumed to denote the completion of seed germination and the beginning of seedling establishment, i.e., the “zero time” for measuring root and shoot growth.

Chlorophyll *a* and *b*, as well as total carotenoids, were determined spectrophotometrically on acetone/water (80/20, v/v) extracts of leaves, according to the method of Lichtenthaler et al. (1982).

Each reported result is the mean $\pm$ SE of at least ten measurements conducted for each of the 3–5 replicated independent experiments.

10.2.3 Laboratory Studies on Root Growth and Morphology

Previously sterilized seeds of *A. thaliana* ecotype Columbia (Col-0) were incubated in the dark at 4°C for 24 h in sterile distilled water. Seeds were then sown onto

square Petri plates (10 cm × 10 cm) containing 40 mL of a sterile half-strength MS medium (Murashige and Skoog 1962), 3% (w/v) sucrose, 0.7% (w/v) plant agar, and final CAT concentrations of 0 (control), 3, 9, or 27 μM obtained by diluting in sterilized 10 mM phosphate buffer pH 6.0 appropriate volumes taken from a sterilized stock 1 mM CAT solution in the same buffer. Petri dishes containing the germinating seeds were incubated at 25°C, with a photoperiod of 16 h light and 8 h darkness, in vertical position, to avoid root penetration in the medium. This allowed an easier measurement of the growth of the primary root and to visualize root hairs and lateral roots. Measurements were done after 5, 8, 11, and 14 days from seeds germination. Image analysis of the *A. thaliana* root apparatus was carried out as stated earlier. The root hair parameters, namely elongation zone, number, and length, were analysed with a stereomicroscope (Olympus MIC-D) at a 98 $\times$ magnification. The shoot analysis was performed by measuring the diameter and counting the number of leaf rosettes.

Each result is the mean $\pm$ SE of at least ten measurements conducted for each of the 3–5 replicated independent experiments.

10.3 Results and Discussion

10.3.1 Root Morpho-Topology in Microcosm-Scale Experiments

Roots can act as CO₂ sink in soil by depositing both dead roots and photoassimilated C in the rhizosphere (Tresder et al. 2005). Conversely, roots can be considered biogenic sources of soil CO₂ through root tissue respiration, and indirectly, stimulation of microbial activity. Beside this source/sink effect for CO₂, roots also exert an indirect action on the rate and extent of SOM mineralization, often denoted as the *rhizo-stimulated SOM-derived CO₂ priming effects*, through the release of easily decomposable C sources (Cheng and Kuzyakov 2005; Dijkstra and Cheng 2007; Cheng 2008).

Root morphology and topology represent primary factors for assessing the functional role of root system in the soil C dynamics. In fact, factors such as root length, surface area, diameter class, and topology, may contribute to CO₂ source potentials, whereas other ones, such as root mass ratio, fineness, and tissue density, are related to CO₂ sink capacity. In this respect, we analysed changes of these root parameters in response to CAT or COM amendments, as added alone or in combination.

The CAT treatment increased the total biomass of maize plants that, conversely, remained unchanged by the COM addition alone. However, the combination of the two treatments produced a synergic effect on total plant biomass (Fig. 10.1). The results mirrored the separate patterns observed for root and shoot dry weight (Fig. 10.2).

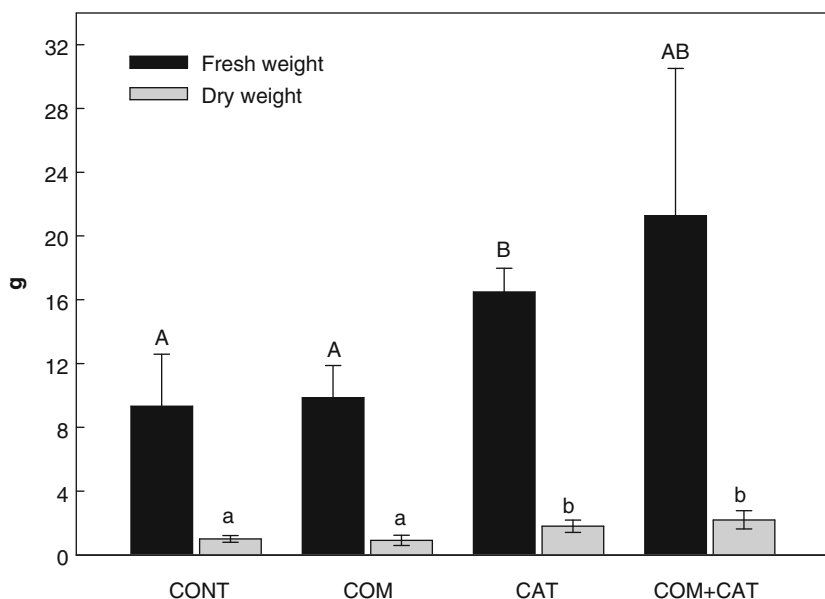


Fig. 10.1 Whole biomass (shoot + root) of maize plants (cv. Cecilia) growing in experimental microcosms filled with: control soil, CONT; compost-amended soil, COM; soil amended with iron–porphyrin, CAT; compost-amended and iron–porphyrin-treated soil, COM + CAT. Values are means ($n = 6$) $\pm$ standard deviation of the mean. Different letters denote statistically significant differences in respect to control (CONT; Tukey’s test, at $p < 0.05$)

Plant root length was increased by COM amendment, though not significantly, while it was not influenced by CAT treatment. As already observed for biomass parameters (Figs. 10.1 and 10.2), a synergic effect was noticed when CAT and COM were added together to the microcosms (Fig. 10.3). Furthermore, COM amendment significantly increased the cumulative length of roots belonging to the fine roots classes (diameter range 0–0.4 mm), and even more so did the COM + CAT treatment (Fig. 10.4a). Conversely, CAT, either alone or in combination with COM, significantly increased the cumulative length of roots within the coarse class (diameter class, >1.2 mm), while larger diameter classes did not respond to COM addition alone (Fig. 10.4b).

Each of the two treatments alone, and even more their combination, tended to increase the root surface area (Fig. 10.5). By dissecting the treatments’ effects for the surface area in respect to diameter classes, a pattern similar to that observed for root length was obtained (see above; compare Fig. 10.6 with Fig. 10.4). Nevertheless, no treatment modified the topological index of the maize root, whose architecture remained of the “herringbone” type ($TI \sim 1$; data not shown).

Beside the morphological root features that potentially increase CO_2 release from soil (soil as a CO_2 source), we also evaluated those components of root morphology which, directly or indirectly, have the potential to enhance carbon storage in soil, thus promoting its role as CO_2 sink. To such aim, we evaluated (1) the root mass

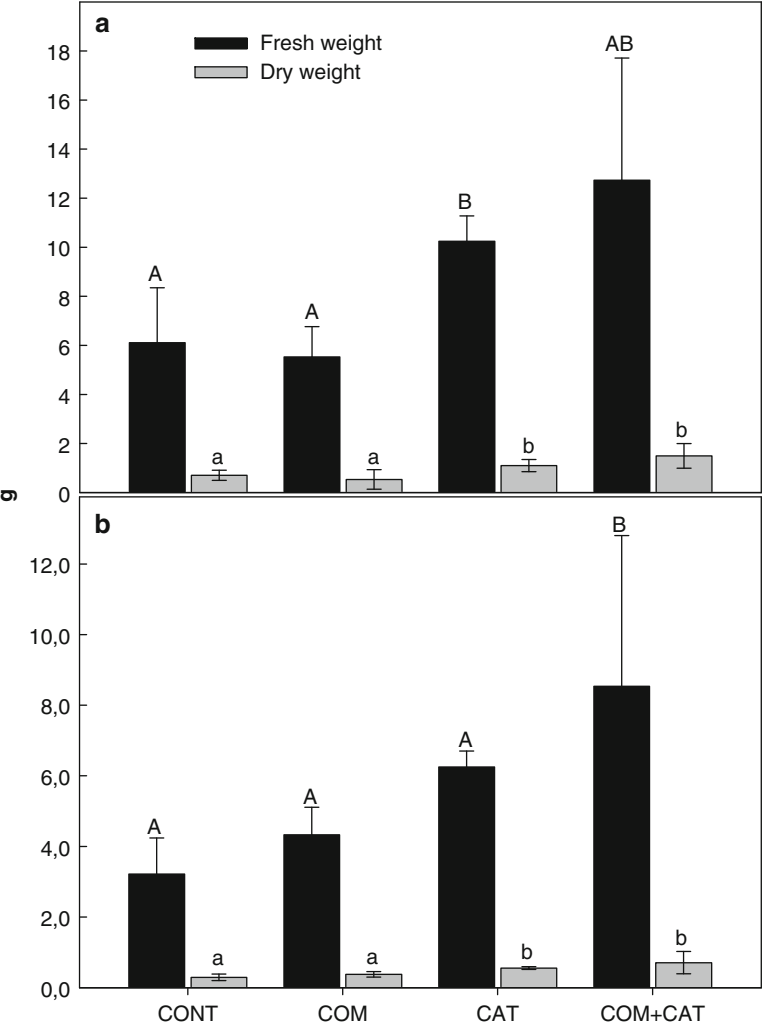


Fig. 10.2 Shoot (a) and root (b) biomass in maize plants in Fig. 10.1. Treatments acronyms and statistics as in Fig. 10.1

ratio (RMR), a component expressing the relative amount of biomass allocated to the root, and (2) the root fineness (F) and the root tissue density (TD), which are the root structural components. In particular, the RMR indicates the plant potential to allocate photosynthetically fixed carbon to soil, whereas the F and TD parameters provide indirect information on the “root biomass quality”, that may have an impact on soil C stabilization after root degradation in soil. It has been observed that TD is negatively related to the root turnover rate (Ryser 1998), while it is positively related to the thickness of root cell walls, to the root sectional area (Wahl and Ryser 2000), and, finally, to the degree of exodermis lignification (Eissenstat and Achor,1999).

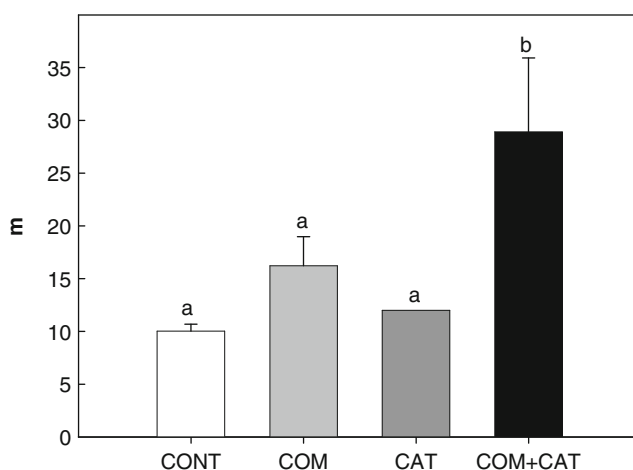


Fig. 10.3 Total root length in maize plants in Fig. 10.1. Treatments acronyms and statistics as in Fig. 10.1

Therefore, both a large TD and/or a low F indicate a high root lignification degree, whose alleged recalcitrance slows down root degradation in soil.

The results reported here suggest that the COM treatment, as compared to CAT, induced a greater biomass allocation to roots (larger RMR in Fig. 10.7). In term of structural components, the CAT treatment strongly reduced the fineness of the root system, implying the prevalence of root axes with large diameters, whereas this same parameter was significantly increased by COM, either alone or in combination with CAT (Fig. 10.8). Remarkably, both single treatments, as well as their combination, reduced root tissue density (Fig. 10.9). This, on the basis of previous considerations, would imply a decreased root recalcitrance to degradation, leading to an accelerated root turnover rate in soils amended with COM and/or treated with CAT.

All together, the above results appeared controversial, by showing a double role as source/sink of both CAT and COM amendments in soil C balance. On one hand, CAT increased the root surface area and length of coarse roots, possibly resulting in an enhanced surface contact between root and soil, that may stimulate CO₂ emission from soil. On the other hand, the biomimetic catalyst reduced the fine roots length and, hence the release of root exudates, implying a decrease of supply to rhizospheric biota of promptly respirable substrates, and resulting in slowing down of CO₂ emission rates from soil.

Likewise, compost amendment behaved similarly to CAT in increasing the root surface area and root length of fine roots, and both may have stimulated plant-derived CO₂ release. However, COM concomitantly favored the increase of fine roots length, with consequent enhanced rhizodeposition, and larger biomass allocation to roots, thereby suggesting that compost may have promoted the role of roots as CO₂ sink in soil. Consequently, the combined CAT + COM treatment exacerbated the contrasting role of the root system to act as either a source or a sink for the soil C balance.

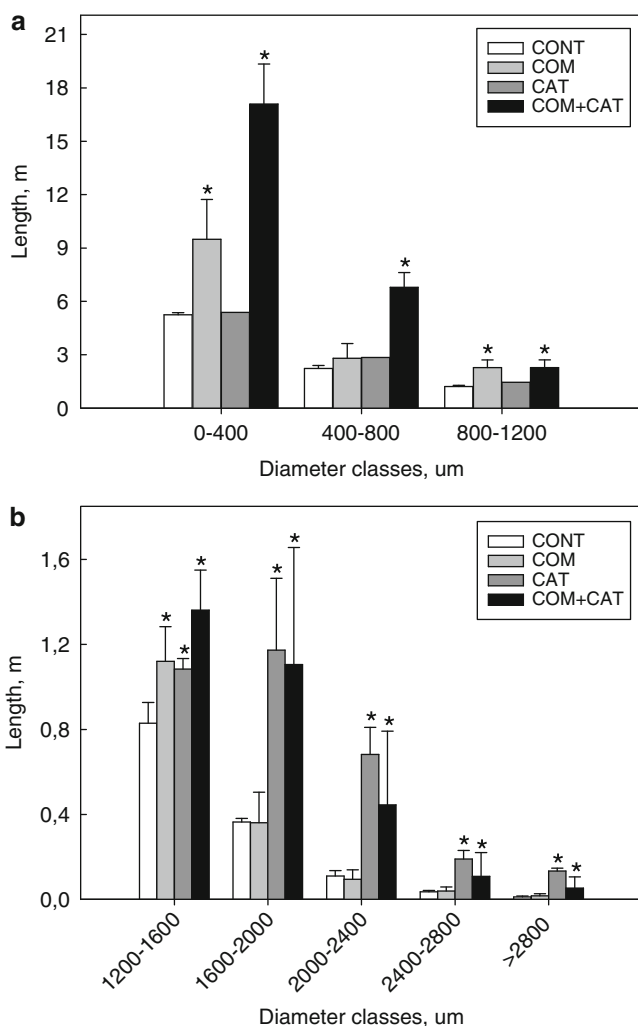


Fig. 10.4 Total length of (a) “fine roots” (<1.2 mm) and (b) “coarse roots” (>1.2 mm) within diameter classes in maize plants as in Fig. 10.1. Treatments acronyms and statistics as in Fig. 10.1. Asterisks indicate a statistically significant difference in respect to control (CONT; Tukey’s test, at $p < 0.05$)

10.3.2 Soil Chemical Properties and CO_2 Emissions from Microcosm-Confined Plant–Soil Systems

Compost amendment markedly affected all selected soil chemical properties. The pH and EC values in COM-amended soils were significantly larger than for unamended soil treatments (Table 10.1), without being affected by plant growth. The TN content was increased by COM, either alone or in combination with CAT

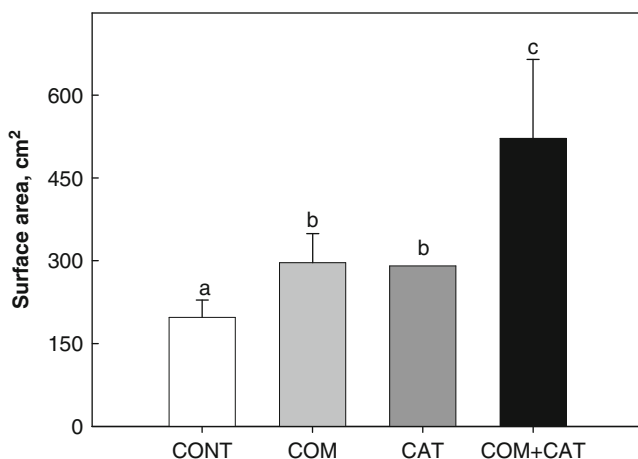


Fig. 10.5 Root surface area in the maize plants as in Fig. 10.1. Treatments acronyms and statistics as in Fig. 10.1

(Table 10.1), while it resulted unaffected by both plant growth and time. Finally, TOC increase in both COM and the CAT + COM treatments was still noticeable at the end of the experiment (76 days), both in unplanted and in planted microcosms (Table 10.1), though TOC was found to slightly decrease (approximately by 5%) over time in all treatments.

According to Sikora and Stott (1996), these results suggest that the whole soil organic C pool does not rapidly respond to environmental changes, unless it is subjected to large amendments or anthropogenic disturbances. Thus, rather than following C-stock variations, we monitored the changes in soil respiration to evaluate the impact of the synthetic iron–porphyrin on soil C dynamics.

Before starting to monitor CO₂ release from soil microcosms, a few weeks lag time was allowed to elapse, in order to avoid any masking action due to the “tillage effect”, that generally occurs after soil physical disturbance (Ellert and Janzen 1999). In particular, the intensive soil mixing occurring during set-up of enclosed model systems is likely to lead to an overestimation of heterotrophic fluxes. As assessed elsewhere for the same clay loam soil used here, approximately 40 days were needed for the tillage effect to subside (Tortorella and Gelsomino 2011).

During the early experimental period, CAT further reduced the already low ($<1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) amount of CO₂ released from unamended soils (Fig. 10.10). At later stages, however, the soil CO₂ efflux from unplanted microcosms sprayed with CAT appeared to be larger than control (Fig. 10.10). The contribution of the rhizosphere-derived C markedly increased soil CO₂ flux. In fact, larger values were recorded in planted microcosms added with CAT.

Even though COM amendment stimulated CO₂ emission from soil (CO₂ efflux $> 1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), CAT addition strongly depressed the compost-induced CO₂ release over the entire experimental period (Fig. 10.11). As observed in the

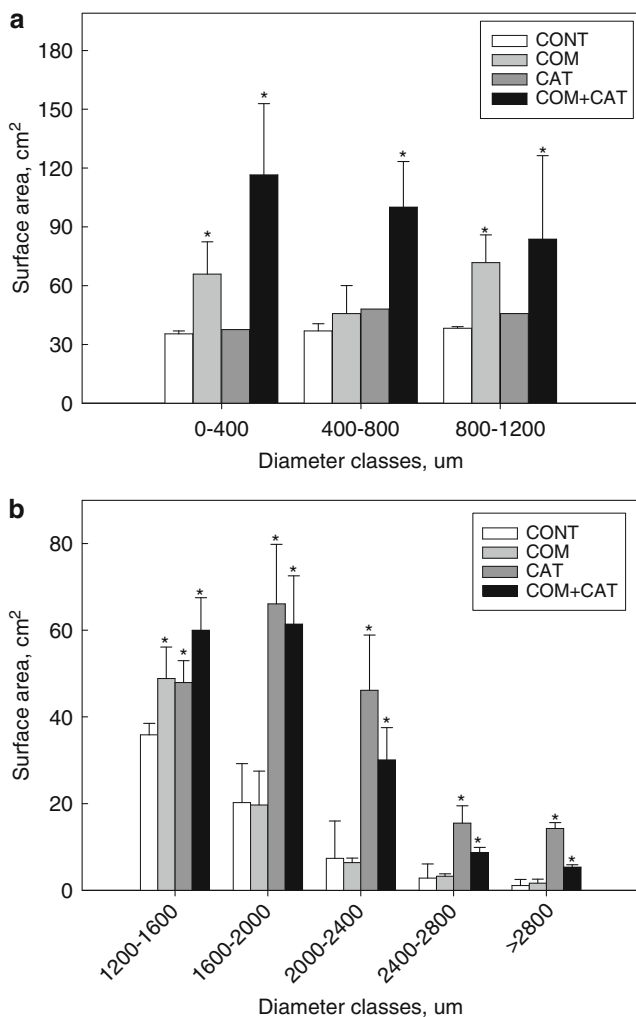


Fig. 10.6 Surface area of (a) “fine roots” (<1.2 mm) and (b) “coarse roots” (>1.2 mm) within diameter classes in maize plants in Fig. 10.1. Treatments acronyms and statistics as in Fig. 10.1. Asterisks indicate a statistically significant difference in respect to control (CONT; Tukey’s test, at $p < 0.05$)

unamended soil, such depressive effect of CAT was reversed in the presence of plants, being the largest flux rates found in the planted COM + CAT treatments (Fig. 10.11).

Before transplanting, a reduced soil CO_2 flux was recorded in CAT-treated microcosms, suggesting that the biomimetic catalyst was active in favoring the stabilization of soil organic compounds against microbial degradation. Although this process could have been still operating after transplanting, the increased plant-induced soil respiration rendered the role of the iron–porphyrin less evident.

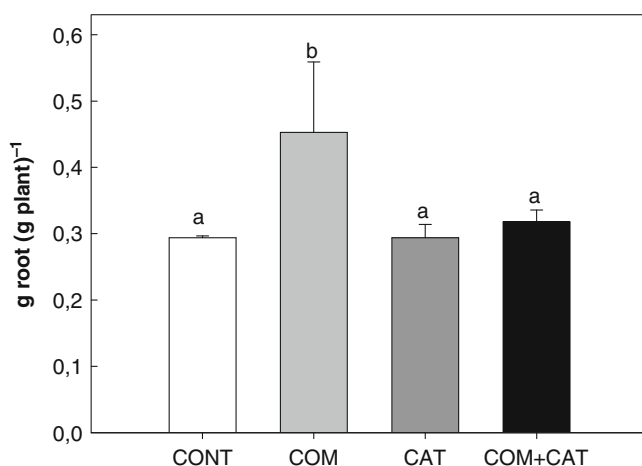


Fig. 10.7 Root mass ratio in maize plants as in Fig. 10.1. Treatments acronyms and statistics as in Fig. 10.1

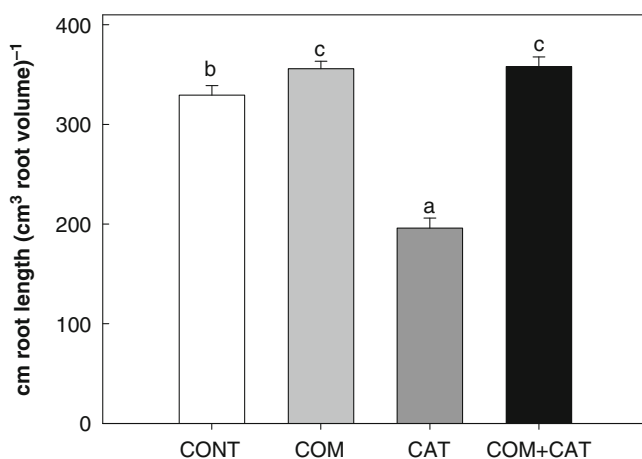


Fig. 10.8 Root fineness in maize plants as in Fig. 10.1. Treatments acronyms and statistics as in Fig. 10.1

An increased soil CO₂ flux from plant-hosting microcosms was not unexpected, as plants may contribute strongly to total CO₂ emission, due to root and rhizo-microbial respiration (Kuzyakov 2006). It is known that a close interaction occurs at the plant–root interface. This affects total soil CO₂ flux, both directly, due to respiration by living root tissues (autotrophic flux component), and indirectly, due to microbial decomposition of dead plant residues, as well as rhizodeposits from living roots (heterotrophic flux component; Cheng and Kuzyakov 2005; Moyano et al. 2007). Here, it appears that iron–porphyrin and maize plant alone exerted an

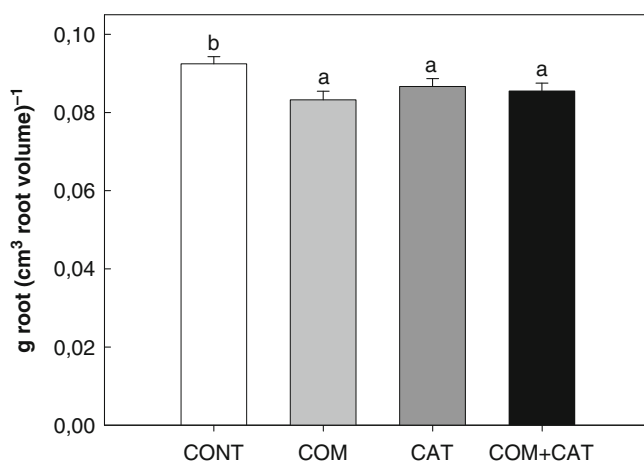


Fig. 10.9 Root tissue density in maize plants as in Fig. 10.1. Treatments acronyms and statistics as in Fig. 10.1

Table 10.1 Changes in pH, electrical conductivity ($EC_{1:2}$), total organic C (TOC), and total N (TN) in the experimental microcosms (unplanted or planted) filled with control soil (CONT), compost-amended soil (COM), soil amended with iron–porphyrin (CAT) or compost-amended and iron–porphyrin-treated soil (COM + CAT) before and after the 76 days experimental period

Treatment	pH			$EC_{1:2}$ (dS m ⁻¹ ; at 25°C)		
	Day 0	Day 76		Day 0	Day 76	
		Unplanted	Planted		Unplanted	Planted
CONT	6.64 (0.08)a	6.66 (0.06)	6.68 (0.04)	0.156 (0.030)	0.221 (0.051)	0.229 (0.053)
COM	7.01 (0.07)*	6.95 (0.04)*	6.99 (0.03)*	0.384 (0.015)*	0.501 (0.087)*	0.472 (0.126)*
CAT	6.69 (0.06)ns	6.66 (0.06)ns	6.71 (0.05)ns	0.187 (0.024)ns	0.192 (0.069)ns	0.209 (0.023)ns
COM + CAT	6.98 (0.07)*	6.98 (0.03)*	6.95 (0.02)*	0.309 (0.075)*	0.415 (0.034)*	0.435 (0.074)*
Treatment	TOC (g kg ⁻¹)			TN (g kg ⁻¹)		
	Day 0	Day 76		Day 0	Day 76	
		Unplanted	Planted		Unplanted	Planted
CONT	19.2 (0.5)	18.1 (0.6)	18.2 (0.4)	1.8 (0.2)	1.7 (0.1)	1.7 (0.2)
COM	22.0 (0.2)*	20.9 (0.7)*	21.0 (0.5)*	2.1 (0.3)*	2.0 (0.1)*	2.0 (0.2)*
CAT	19.1 (0.6)ns	18.4 (0.4)ns	18.1 (0.4)ns	1.8 (0.1)ns	1.7 (0.1)ns	1.6 (0.1)ns
COM + CAT	21.5 (0.4)*	20.8 (0.5)*	20.7 (0.5)*	2.1 (0.1)*	1.9 (0.2)*	1.8 (0.2)ns

^a Values are means (n = 6) with standard deviation in brackets; for column means, asterisks denote significant differences respect to the control (CONT) at the $0.01 < p < 0.05$ level (Tukey's test); ns, not significant at the chosen level of statistical significance.

opposite action on soil respiration. The combination of the two factors, however, unexpectedly increased soil CO₂ emission.

A possible explanation of such unforeseen outcome is that the maize root system might have acted as a secondary target in the CAT treatment. This could have changed certain root morphological features, thereby determining an altered pattern of soil respiration rates. In fact, although maize roots from CAT and CONT

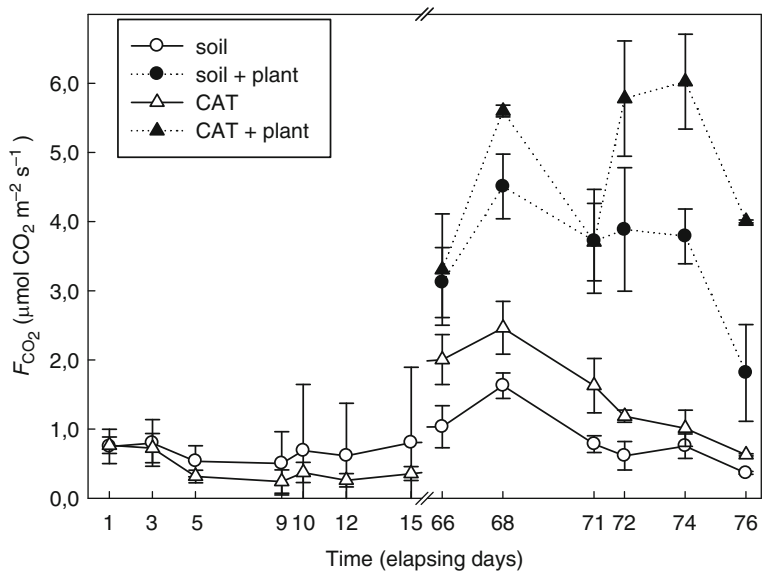


Fig. 10.10 Soil CO₂ efflux from compost-free microcosms. Treatments acronyms and statistics as in Fig. 10.1

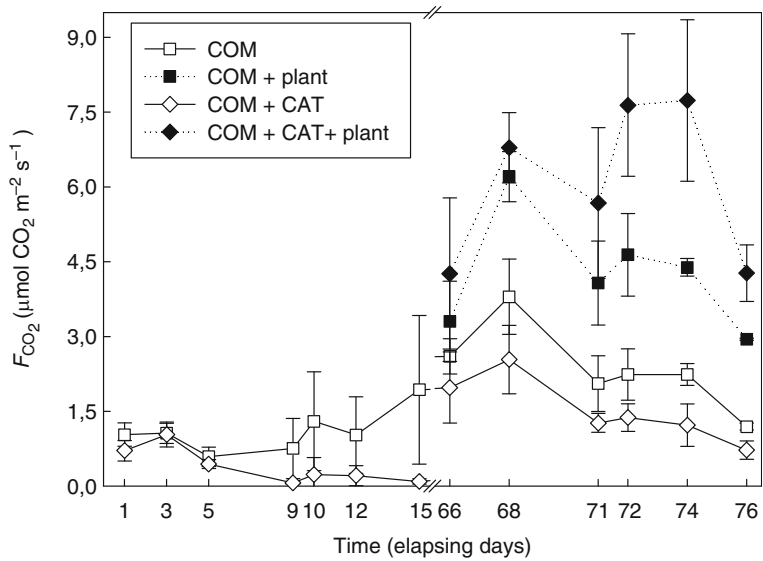


Fig. 10.11 Soil CO₂ efflux from compost-amended microcosms. Treatments acronyms and statistics as in Fig. 10.1

treatments showed similar length- and mass ratio values, those for the former were coarser than for the latter (lower fineness and higher length in the >1.2 mm diameter classes). Noticeably, coarse roots are able to penetrate hard soil layers (Clark et al. 2003) and reorganize soil structure by alternate formation and destruction of soil aggregates (Haynes and Beare 1997). In this respect, Helal and Sauerbeck (1984, 1986) and then Cheng and Kuzyakov (2005) suggested “a positive priming effect of living roots on SOM decomposition by soil aggregate destruction”. It may thus be inferred that a coarser maize root system induced by the biomimetic catalyst favored the breakdown of soil macroaggregates, thus exposing physically protected SOM to microbial attack.

Interestingly, a stimulatory effect on soil respiration due to CAT was also observed in the CAT + COM/maize plant treatment, suggesting that compost addition enhanced the response of the CAT–maize root interaction. However, stimulation of soil respiration by compost addition became statistically significant only at a late experimental stage, when compost was a single source of variability. An increase of soil respiration following compost addition had been previously observed by Borken et al. (2002) and Bastida et al. (2008), who hypothesized that such increase may be caused by (1) microbial decomposition of applied compost, (2) increased OM decomposition rate, (3) increased root respiration. Regarding this latter aspect, we noticed that the maize root system exposed to CAT + COM treatment was longer and characterized by more numerous fine and coarse roots than for control. We may speculate that morphological root traits of CAT + COM-treated plants could be functionally related to soil CO₂ flux. In fact (1) a greater root length is related to enhanced efficiency of plant root systems to explore larger soil volumes (Ryser 1998) and, consequently, enhanced rhizo-microbial respiration may be inferred, (2) a greater proportion of fine roots has the potential to increase both autotrophic and heterotrophic microbial respiration through an increased release of root-derived C inputs (Pregitzer et al. 2000, 2008), (3) the disaggregating effect of the coarse portion of the maize root systems may contribute to soil respiration, as discussed earlier. Hence, root morphological changes induced by the combined CAT + COM treatment may be responsible for an increased soil CO₂ flux.

As our results appear to suggest that a direct interaction between plant root system and biomimetic catalyst overcomes, or even contrasts, the catalyst action in stabilizing soil C, further studies were undertaken to understand if and how the iron–porphyrin per se could influence seed germination, as well as seedling establishment and growth. The main results of such studies are presented as follows.

10.3.3 Direct CAT Effects on Seed Germination and Post-germinative Processes

The CAT treatment had no significant effect on the germination of both garden cress and carrot seeds (data not shown). However, CAT appeared to stimulate

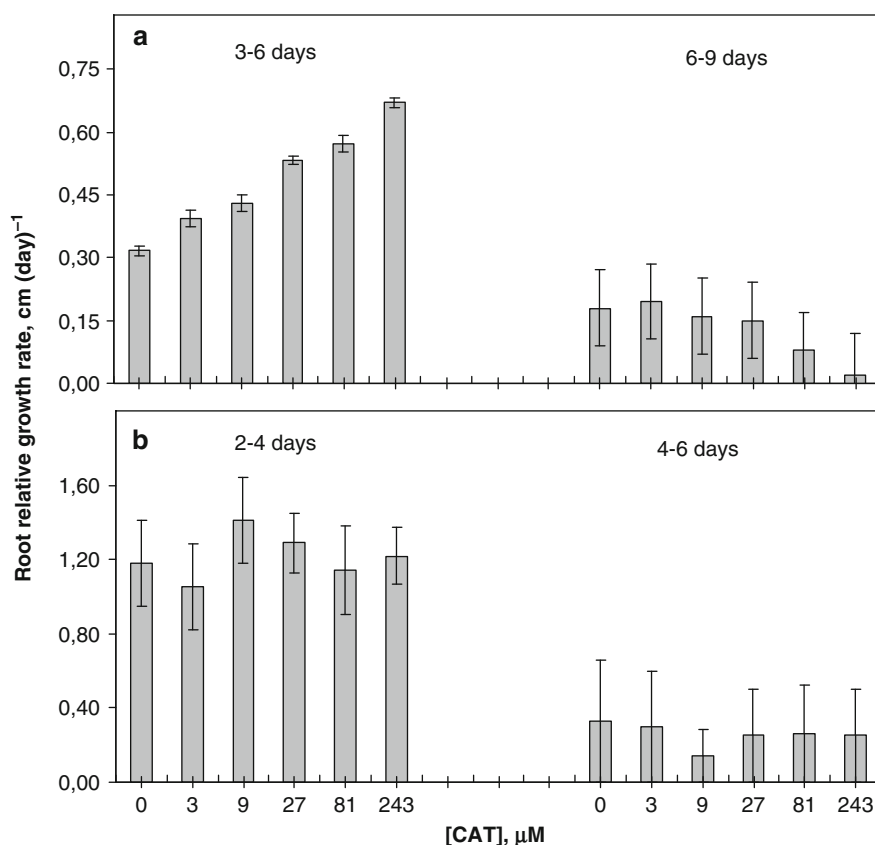


Fig. 10.12 Root growth in carrot (**a**) or garden cress (**b**) plantlets, grown for 9 or 6 days in the dark, respectively, as affected by the presence of increasing concentrations of CAT iron–porphyrin

radicle elongation in carrot seedlings, but not in garden cress, depending on concentration until the 6th day of growth (Fig. 10.12).

Since CAT-treated garden cress leaflets were visibly greener than control, exploratory experiments on the photo-induction of chlorophyll (Chl) and accessory pigment were run. In seedlings grown for 6 days in the dark and then exposed to 5 h of light, CAT increased the contents of total Chl (Fig 10.13) as well as carotenoids (Fig. 10.14) as a function of concentration, except for the seedlings treated with the largest CAT concentration (243 μM), whose Chl values were similar to control (Fig 10.13).

When the above photo-induced garden cress plants were exposed to a subsequent 20-h dark period, the Chl content decreased in all treatments, even though it remained larger in CAT-treated seedlings (except for CAT = 243 μM; Fig. 10.13). Carotenoids contents, on the other hand, were not reduced by exposure to dark, both for control and CAT up to 27 μM (Fig. 10.14).

Six days of germination under continuous light did not change the content of pigments in garden cress leaflets (Fig.10.15), even though Chl tended to decrease as

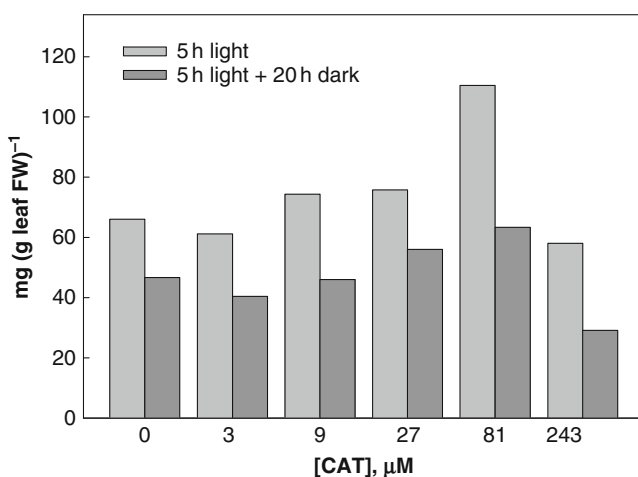


Fig. 10.13 Effects of increasing concentrations of CAT iron-porphyrin on total chlorophyll of garden cress plantlets grown for 6 days in the dark and then exposed to two different light/dark regimes

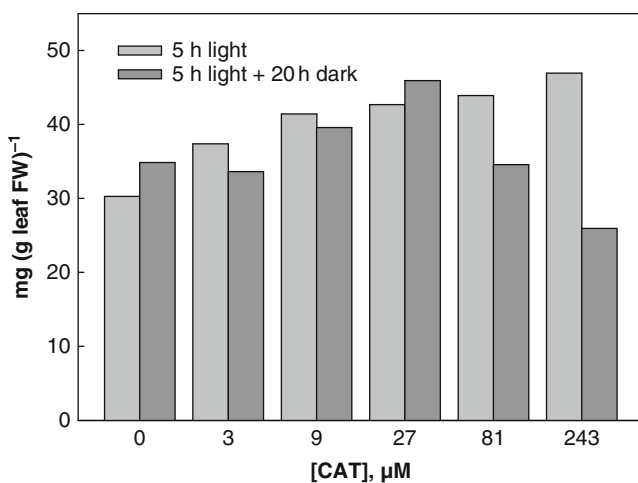


Fig. 10.14 Effects of increasing concentrations of CAT iron-porphyrin on total carotenoids of garden cress plantlets grown for 6 days in the dark and then exposed to two different light/dark regimes

the CAT concentration increased. Under continuous light, the smallest CAT concentrations, but not the largest, promoted both radicle elongation (Fig. 10.16) and seedlings' fresh weight in respect to control (+10%; Fig. 10.17).

Light is directly required for Chl synthesis at the level of enzymatic reduction of protochlorophyllide (Pchl_{id}) to chlorophyllide (Chl_{id}), a direct precursor of Chls *a* and *b*. In angiosperms, such light- and NADPH-dependent reduction of the double

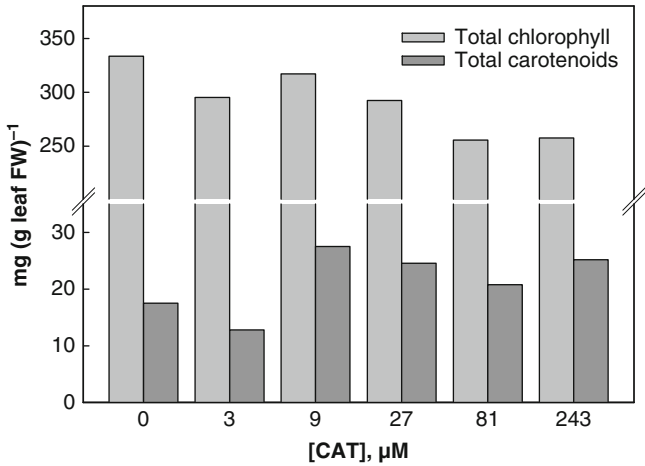


Fig. 10.15 Effects of increasing concentrations of CAT iron–porphyrin on photosynthetic pigments of garden cress plantlets grown for 6 days under continuous light

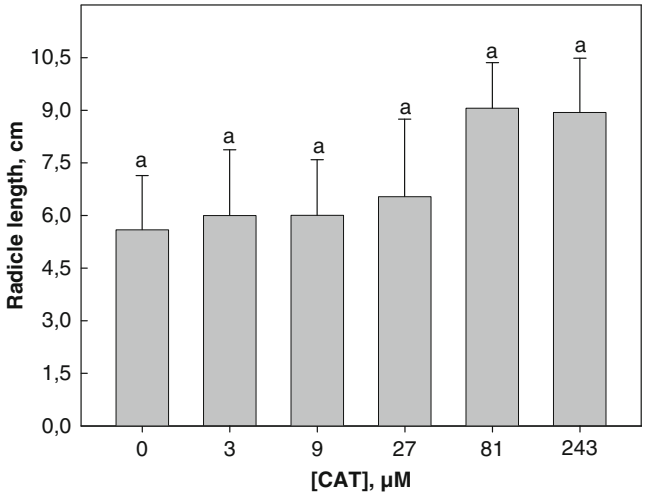


Fig. 10.16 Effects of increasing concentrations of CAT iron–porphyrin on the root growth of garden cress plantlets grown for 6 days under continuous light

bond is carried by the NADPH-protochlorophyllide oxidoreductase (POR; EC 1.3.1.33). The binding of Pchl_{id}e and NADPH to the POR polypeptide leads to an organized ternary complex. Because of its light dependency, POR is not considered simply as an enzyme, but rather as a plastid-specific photon sensor, triggering pigment biosynthesis and membrane reorganization during the transformation of etioplasts to chloroplasts, and leading to conversion of cotyledons from storage organs to photosynthetically competent structures (Heyes et al. 2007).

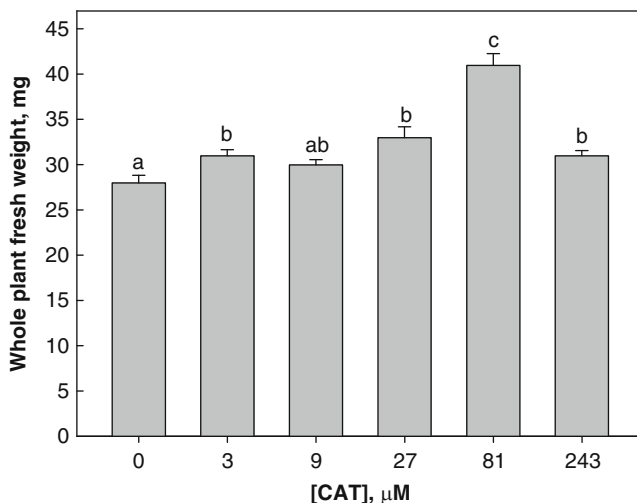


Fig. 10.17 Effects of increasing concentrations of CAT iron-porphyrin on the fresh weight of garden cress plantlets grown for 6 days under continuous light

Two different species of Pchl_{ide} have been identified. One of these is aggregated to the POR ternary complex and is termed photoactive because of its immediate reduction to Chl_{ide} *a* following a single millisecond flash illumination. It has a fluorescence emission maximum at 655 nm (Pchl_{ide}-F655). A second Pchl_{ide} form is non-photoactive, with an emission maximum at 632 nm (Pchl_{ide}-F632). It was supposed that Pchl_{ide}-F632 is non-photoconvertible because it forms Pchl_{ide} aggregates, which are not bound to the POR complex (Armstrong et al. 2000).

It has been reported that high levels of the photoactive Pchl_{ide} may protect plants from photodamage under conditions of high irradiance. In fact, the aggregated structure of Pchl_{ide}-F655 and its association to the POR polypeptide might ensure an effective NADPH supply, thus channeling excess excitation energy towards photoreduction of Pchl_{ide} to Chl_{ide}, rather than to photo-oxidative processes (Skribanek et al. 2000).

Our observations lead us to speculate that the iron-porphyrin based catalyst, structurally similar to the tetrapyrrole intermediates in the chlorophyll biosynthesis, might be integrated in the POR ternary complex, thus acting as a photoactive Pchl_{ide} and promoting the formation of Chl_{ide}. At the largest CAT concentration (243 μM), however, an excessive accumulation of tetrapyrrolic molecules might occur, thus increasing the risk of electron abstraction and radical formation upon excitation by light. Such light-induced radical character could have been transferred to molecular oxygen, leading to a hyper-production of reactive oxygen species (e.g., singlet oxygen) with a consequent enhanced risk of oxidative damage. This may explain the depressive effect of large CAT concentrations on the content of photosynthetic pigments.

All together, the above observations suggest not only a direct “contact effect” of CAT on radicle growth, but also an uptake of the molecule by roots and its translocation to shoots with an influence on Chl metabolism. Should ad hoc biochemical and physiological studies might substantiate the preliminary evidence reported here, it is tempting to consider synthetic water-soluble iron–porphyrins as potentially useful molecules under typical stressful conditions in Mediterranean environments, due to their potential in severely affecting structure and function of light-intercepting machinery in plants, such as photo-oxidative stress and lime-induced chlorosis.

10.3.4 Direct CAT Effects on Root Growth and Morphology

In the *Arabidopsis* model species, neither germination rate nor germination time was changed by the presence of CAT (data not shown). Conversely, the fresh weight of the maize plants after 14 days of exposure to CAT was reduced, as compared to untreated plants (Table 10.2). Such reduction was considerable for both roots and shoots, being larger in the former, and directly correlated to CAT concentration in the medium (Fig. 10.18). At the larger CAT concentrations (9 and 27 μM), the shoot/root ratio was significantly altered in comparison to control

Table 10.2 Effect of varying concentrations of the CAT iron–porphyrin (μM) on fresh weight (FW, mg) allocation in *Arabidopsis* seedlings

CAT	Whole plant FW	Leaf FW (1)	Root FW (2)	(1)/(2)
0	18.44 $\pm$ 1.16a	13.24 $\pm$ 0.68a	5.20 $\pm$ 0.58a	2.71 $\pm$ 0.26a
3	11.07 $\pm$ 1.12b	7.41 $\pm$ 0.69b	3.66 $\pm$ 0.64b	2.43 $\pm$ 0.30a
9	5.74 $\pm$ 0.68c	4.43 $\pm$ 0.53c	1.31 $\pm$ 0.19c	3.73 $\pm$ 0.44b
27	2.69 $\pm$ 0.38d	2.00 $\pm$ 0.25d	0.69 $\pm$ 0.14d	3.37 $\pm$ 0.34b

Different letters denote statistically significant differences ($p \leq 0.05$) respect to control

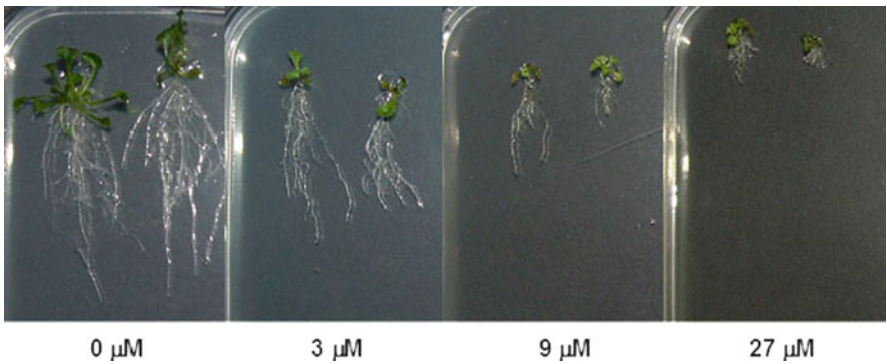


Fig. 10.18 *Arabidopsis* plants exposed for 14 days to different concentrations of CAT iron–porphyrin

(Table 10.2), thus suggesting a biomass redistribution, following probable changes in internal translocation and availability of nutrients taken up by the roots.

The analysis of the root morphology showed a negative CAT effect on the total root length (primary + laterals) after 8 days of growth, at all concentrations (Fig. 10.19). In particular, treatments with 3 and 9 μM CAT caused a 17% and a 75% root length inhibition, respectively, while at a CAT concentration of 27 μM the root length reduction reached up to 85%, as compared to control. The length of the primary root was not affected by 3 μM CAT, whereas it decreased by 50% in plants

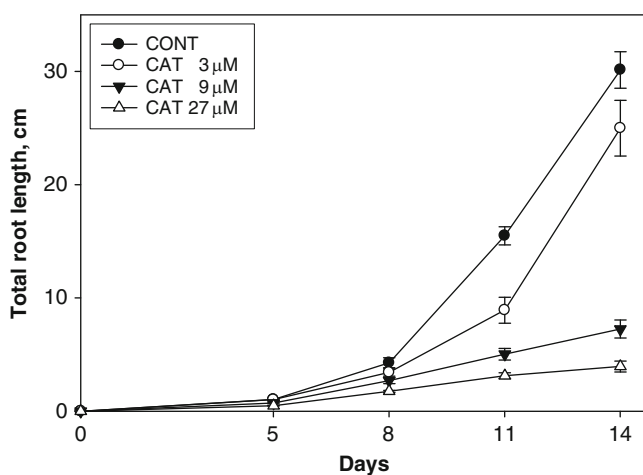


Fig. 10.19 Total root length in *Arabidopsis* plants of Fig. 10.18

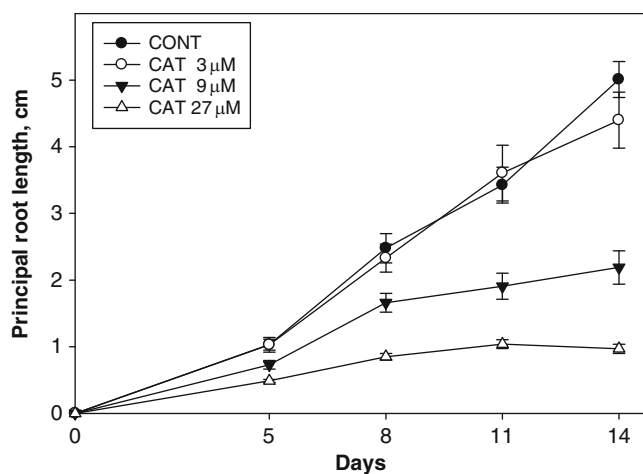


Fig. 10.20 Primary root length in *Arabidopsis* plants of Fig. 10.18

grown with 9 μM CAT and its growth was totally arrested after 14 days with 27 μM CAT in the medium (Fig. 10.20).

It was however the growth of lateral roots, rather than primary ones, to be most inhibited by CAT (see Fig. 10.20 versus Fig. 10.21). However, despite such growth inhibition, 9 and 27 μM CAT caused an anticipated emergence of lateral roots, which became already visible after 5 days from germination, whereas 8 days were required for lateral root emergence in control plants and for those exposed to only 3 μM CAT (data not shown).

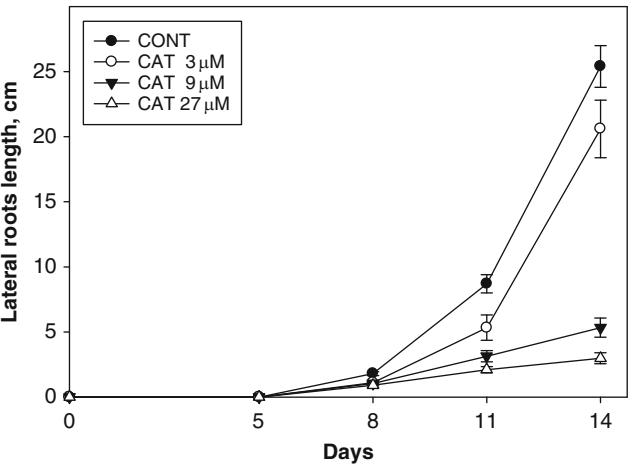


Fig. 10.21 Length of lateral roots in *Arabidopsis* plants of Fig. 10.18

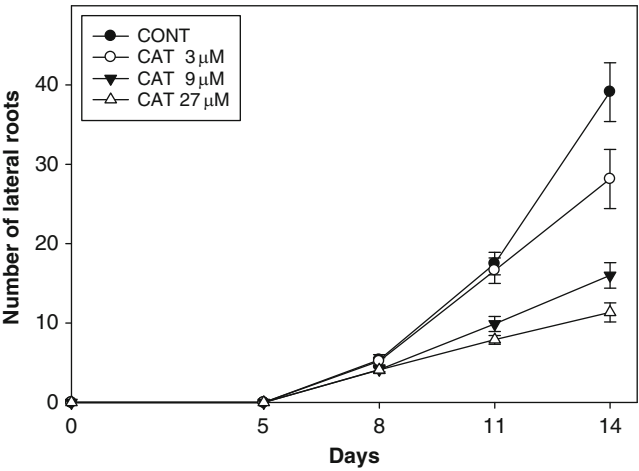


Fig. 10.22 Number of lateral roots in *Arabidopsis* plants of Fig. 10.18

An inhibitory action of CAT was also observed on the number of lateral roots, though less substantial than on their length (Fig. 10.22). This affected the root density, i.e., the ratio of the number of lateral roots over the length of primary roots, that appeared to be larger in plants treated with 9 and 27 μM CAT than in control (Fig. 10.23).

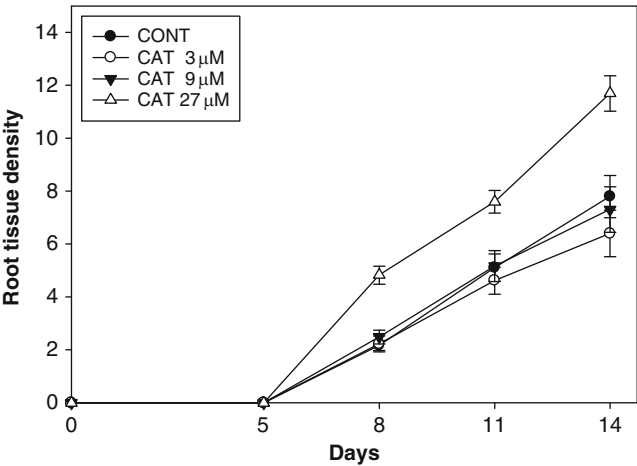


Fig. 10.23 Root density (ratio of number of lateral roots over length of primary root) in *Arabidopsis* plants of Fig. 10.18

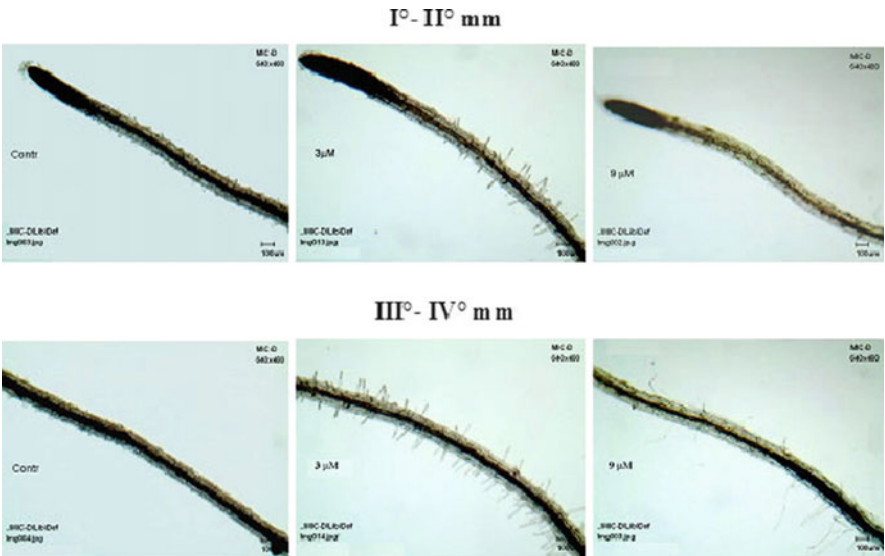


Fig. 10.24 Primary root of *Arabidopsis* after 8 days of growth at different concentrations of CAT iron-porphyrin

Table 10.3 Root hairs development in *Arabidopsis* seedlings as affected by varying CAT iron–porphyrin concentrations (μM)

CAT	Elongation zone	Hairs number			Hairs length (μm)		
		1°mm	2°mm	3°mm	1°mm	2°mm	3°mm
0	1.76 ± 0.64a	0	15 ± 1.29	14.50 ± 2.33	0	40.43 ± 3.09	40.43 ± 3.09
3	1.24 ± 0.08c	0	19.67 ± 4.37	21.33 ± 2.03	0	50.95 ± 5.35	80.30 ± 10.04
9	1.61 ± 0.31a	0	5 ± 2.89	5.67 ± 0.33	0	78.65 ± 3.80	103.55 ± 6.16

Different letters denote statistically significant differences ($p \leq 0.05$) respect to control

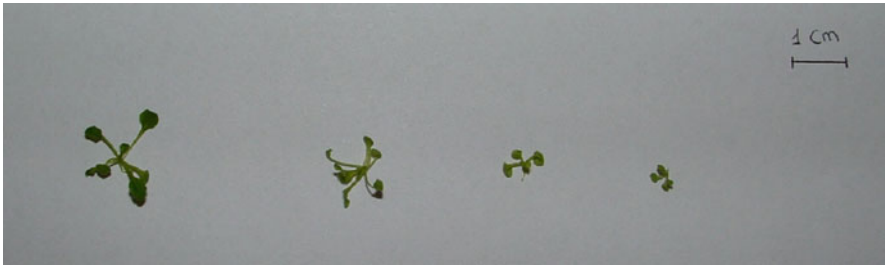


Fig. 10.25 Rosettes of *Arabidopsis* plants exposed to varying concentrations of CAT iron–porphyrin for 14 days

Microscopic analysis (Fig. 10.24 and Table 10.3) revealed that, even at 3 μM CAT, the elongation zone between the primary root apex and the point of emerging root hairs was significantly shorter, and root hairs were more numerous and longer than in control. Conversely, exposure to 9 μM CAT caused a reduction in the number of root hairs, but they were longer than for both control plants and those treated with 3 μM CAT. After 8 days in the presence of 27 μM CAT, the development of the primary root was so strongly inhibited that evaluation of root hairs was not feasible. All together, the above results suggest that comparatively low concentrations of CAT affect the pattern of root hair growth, rather than arresting their growth.

Moreover, we found that CAT induced changes in the root architecture of *Arabidopsis* seedlings, at all tested concentrations. This is considered a general adaptive plants response vis-à-vis the changes occurring in their growth environment. The formation of lateral roots from primary root increases the plant capacity for soil exploration, and root hairs are directly involved in the uptake of water and nutrients. Both processes are influenced by several factors and this dynamic root development (plasticity) represents the main survival strategy for non-motile organisms such as plants.

However, a negative CAT effect was observed on the shoot development of *A. thaliana* seedlings (Fig. 10.25). This may have been either the consequence of inhibitory effects on the root system or a specific CAT effect on the aerial part, following possible absorption and translocation of the iron–porphyrin molecule. With 9 and 27 μM CAT, the number of leaves was reduced by 58 and 51%, respectively, as compared to control. The rosette diameter was lowered by 35% with 3 μM CAT and by 70–80% at larger concentrations. This indicates that CAT

Table 10.4 Rosette morphology in *Arabidopsis* seedlings as affected by varying CAT iron–porphyrin concentrations (μM)

CAT	Mean number of leaves in each rosette	Mean rosette diameter (mm)
0	$7.55 \pm 0.17\text{a}$	$20.77 \pm 0.74\text{a}$
3	$6.91 \pm 0.21\text{a}$	$13.27 \pm 0.75\text{b}$
9	$4.40 \pm 0.27\text{b}$	$7.90 \pm 0.43\text{c}$
27	$3.82 \pm 0.12\text{b}$	$6.73 \pm 0.38\text{d}$
Different letters denote statistically significant differences ($p \leq 0.05$) respect to control		

mainly affected leaf extension, rather than leaf number, as suggested by values of the rosette diameter reported in Table 10.4.

10.4 Conclusions

Porphyrins and metal–porphyrins represent a vast class of chemically reactive compounds that are being used in several biotechnological applications (Smith 1975; Lesage et al. 1993). In particular, a synthetic iron–porphyrin was recently used as a biomimetic catalyst to promote oxidative coupling reactions in soil, in order to increase both the chemical protection of organic C pool (Šmejkalová et al. 2006; Fukushima et al. 2010; Piccolo et al., 2011) and the removal of toxicants from the soil environment (Hahn et al. 2007). Within this context, the functional transformation of soil humic matter is a novel and, apparently, effective strategy in the current strive to control organic matter in ecosystems.

The results from microcosm experiments presented here suggest that, when applied to bare soil, the synthetic iron–porphyrin is able to reduce CO_2 emission from soil. On the contrary, compost amendments stimulated CO_2 emission from soil. However, the combined addition of compost with the iron–porphyrin strongly depressed the compost-induced CO_2 release over the entire experimental period.

In planted microcosms, the contribution of the rhizosphere-derived CO_2 flux markedly increased the total soil respiration and the biomimetic catalyst addition further stimulated CO_2 release from soil. This finding suggests that iron–porphyrin, growth of maize root, and CO_2 release are functionally interconnected, though the mechanism of such interconnection is not clear yet (see Chaps. 4, 6, and 9). The increased total soil respiration observed in planted systems may be due to a larger contribution of rhizosphere-derived CO_2 fluxes, as a consequence of a secondary action of iron–porphyrin on plant root systems. However, it is also possible that the occurred catalyst-assisted photo-polymerization of SOM molecules altered the humic conformation towards an increased bio-accessibility of previously protected humic molecules. A deeper knowledge of the complex relationship existing among biomimetic catalyst, maize root, and CO_2 fluxing mechanisms may be better achieved by further testing soil–plant microcosms filled with contrasting soil types and SOM qualities.

Laboratory studies concerning direct effects on model plant species, by using CAT concentrations similar to those used for microcosm experiments, revealed a complex pattern of rate-dependent and, remarkably, species-specific responses, as observed in both root systems and aerial plant parts. This, together with the ostensibly promoting effects on the synthesis of photosynthetic pigments, encourages to imagine a potential for an *in planta* uptake and translocation of the iron–prophyrin in a perspective of widening its potential usefulness for a range of possible applications, even beyond soil-related biotechnologies.

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Chapter 11

New Modeling Approach to Describe and Predict Carbon Sequestration Dynamics in Agricultural Soils

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Abstract The contribution of agro-ecosystems to carbon sequestration in the form of soil organic matter (SOM) is increasingly considered as a mitigating factor for climate change. The ecosystem carbon storage depends on the balance between C inputs and outflows due to SOM breakdown. SOM decomposition has been reported as mostly affected by temperature and water availability, at global and regional scale, and by C quality at local scale, where climate can be considered relatively uniform. In this work, a new model of SOM decomposition is presented. The SOMDY model is based on an advanced description of SOM chemical quality by ^{13}C -CPMAS NMR instead of traditional C/N ratio. The model includes also the effects of physical aggregation of organic matter. SOMDY was calibrated on CO_2 emission data from extensive field experimental measurements. The simulation results showed the model capability to predict SOM changes during decomposition processes, including the effects of addition of organic amendments (e.g., compost applications, crop residual burial), as well as the impact of different tillage practices on the physical structure of soil aggregation.

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11.1 Introduction

The potential of agro-ecosystems to absorb large quantities of atmospheric carbon dioxide through carbon sequestration in the form of soil organic matter (SOM) is widely being put forward as one of the mitigating options for climate change (Post et al. 2004). In these man-controlled ecosystems, only a limited fraction of net primary production is yearly delivered to soil as dead organic matter, while a significant amount of organic C enters the soil from external inputs as organic amendments (cover crop, manure, compost, etc.).

However, the ecosystem carbon storage depends not only on C inputs, but also on its outflows, which are controlled by decomposition and mineralization processes. Organic matter decomposition rates are affected by climatic variables, litter quality, and soil disturbance and they change according to the level of incorporation of organic materials into the soil system. Temperature and water availability are considered the most important factors acting at global and regional scale (Aerts 1997; Incerti et al. 2011), while at local scale, when climate can be considered uniform, organic matter decay rate is mostly affected by C quality, i.e., the susceptibility of the substrate to be transformed by decomposers (Meentemeyer 1978).

The definition of organic matter quality in terms of organic chemical composition (Swift et al. 1979) is operationally difficult because litter contains several organic compounds with different susceptibility to decomposition (e.g., lignin, tannins, cellulose, organic acids, amino acids, simple sugars, humic substances) and several inorganic elements (e.g., N, P, S) whose relative fractions vary with decay stage (Rovira and Vallejo 2007). During the last decades, a substantial effort has been made to search effective indicators of organic matter quality, capable to provide reliable predictions of decay rate. The traditional approach has been based on the assessment of selected characteristics to identify parameters or indexes correlated with decay rates, and thus useful for predictive purposes (Meentemeyer 1978; Melillo et al. 1982). Several works reported consistent negative correlations with organic matter decay rate for carbon to nitrogen content ratio (C/N) (Taylor et al. 1989) and, limitedly to litter, for lignin to nitrogen content ratio (Lignin/N) (Melillo et al. 1982). Consequently, C/N and lignin/N ratios are extensively used in most C-cycle models, as descriptors of SOM and litter quality to control mass loss rate (Burke et al. 2003; Adair et al. 2008).

However, a more complex system develops as organic matter enters into soil, due to its interactions with the soil mineral fraction (Piccolo 1996). Consequently, a reliable description of SOM dynamics cannot be based only on organic matter quality indicators, but it should include OM interactions with soil mineral constituents (i.e., sand, silt, and clay fractions).

Moreover, it is widely known that soil disturbance by tillage practices greatly affects SOM stocks, due to their accelerated decomposition. Intensive tillage is widespread in modern agro-ecosystems to prepare seedbed, to incorporate mineral fertilizers, organic amendments and crop residues into soil, to reduce compaction, and effectively control weeds (Conant et al. 2007). Nevertheless, the diffusion of

mechanical tillage is believed to be a primary cause of the historical SOM loss in agro-ecosystems, following the conversion of natural soil to agriculture (Drinkwater et al. 1998). Intensive soil tillage increases soil gas exchanges and distribution of organic residues through the soil profile. However, while the release of mineral nutrients is favored by an enhanced SOM decomposition caused by deep tillage, the stock SOM is in turn progressively depleted. As opposite to traditional tillage, the practice of conservative tillage is indicated by many studies to be likely to increase C sequestration in soil (Reicosky 2003). However, the evidence that reduced tillage promotes C sequestration are highly variable (Baker et al. 2007). Recently, Bonanomi et al. (2011a) reported a large decrease of C stock (-24%) from soils under intensive cultivation (more than six tillage treatments per year), as compared to low-input tree orchards (less than two yearly treatments).

In this regard, a considerable modeling effort has been done in last decades to mathematically describe SOM decomposition processes (for an extensive review, see Shibu et al. 2006), which are invariably included in most biogeochemical models simulating carbon dynamics and other ecosystem processes from annual to millennium scale. The main examples are CENTURY (Parton et al. 1994), ROTHAMSTED (RothC) (Coleman and Jenkinson 1996), LPJ (Sitch et al. 2003), and the Biome- (Hunt et al. 1996) and Forest- (Running and Gower 1991) BGC (Bio Geochemical Cycles) models. These detailed models are often used for simulations at regional or global level (e.g., Gholz et al. 2000), whereas their application at lower scale, particularly in Mediterranean agro-ecosystems, is much less reported (e.g., Hoff et al. 2002). For instance, models of SOM, such as CENTURY and RothC, have been developed and are now worldwide applied to evaluate the effects of various agricultural management practices on soil C stock.

In these models, litter and soil organic matter are usually divided in different compartments (early model in Hunt 1977; Jenkinson and Rayner 1977; Minderman 1968). In particular, litter is classified using three different criteria: chemical, kinetic, and functional. The chemical approach defines pools by difference in litter chemical components (e.g., Minderman 1968). This approach has the advantage that chemical compounds (i.e., water-soluble carbohydrates, holocellulose, and lignin) can be analytically determined (Allen 1989; Rowland and Roberts 1994). The kinetic approach defines pools according to their decomposition rate. For example, the Roth-C model (Jenkinson and Rayner 1977) distinguishes two kinetically defined pools of plant litter: decomposable (DPM) and resistant plant materials (RPM). The functional approach describes plant litter as composed by metabolic (labile) and structural (i.e., resistant) pools. According to this method, cell components degrade somewhat independently from the physical structure of plant material. Examples of this type of litter characterization are found in CENTURY (Parton et al. 1987, 1988) and GRAZPLAN (Hunt 1977) models.

Different models represent SOM as distributed in two or more pools with different turnover rates. In this way, litter inputs are assigned to SOM pools according to the initial quality of either structural or metabolic litter components. Generally, current approaches are either box models, in which organic matter compounds with similar dynamics are pooled (e.g., Century; Parton et al. 1987)

or continuum models, which track degradation of litter quality during decomposition of substrates which steadily decompose (e.g., Ågren and Bosatta 1996). Recycling of C from older pools through microbial biomass is allowed in both model types.

In contrast, models specifically designed for application to agro-ecosystems tend to be used for single season simulations, using daily changes in nutrient and water availability to constrain crop growth and development (DNDC: Li et al. 2003; DayCent: Del Grosso et al. 2001; CANDY: Franko et al. 1995). In general, whether they are from an agricultural or ecological viewpoint, models developed primarily for estimating SOM storage include SOM quality indicators based on the C/N ratio. In spite of research efforts, the reliability of such indicator is not free of uncertainty. For instance, Berg and McClaugherty (2008) suggest that using C/N ratio to predict decay rate throughout the decomposition process should be avoided, because, irrespectively of its initial value, C/N progressively decreases as C is lost through respiration, while N is immobilized in microbial biomass (Bonanomi et al. 2010).

In the last decade, chemical throughput methods as pyrolysis-gas chromatography/mass spectrometry (Huang et al. 1998), near infrared reflectance spectroscopy (Gillon et al. 1999), and solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy either as such (Piccolo et al. 1990; Kögel-Knabner 2002) or in combination with chemometry (Smejkalova et al. 2008) have been applied to characterize organic matter at molecular level. In particular, ^{13}C -CPMAS NMR has been proven useful to provide an overview of the total organic chemical composition of complex matrices, such as soil organic matter (Kögel-Knabner 2002; Preston et al. 2009; Bonanomi et al. 2011b). However, such novel experimental applications have not yet been exploited by current modeling implementation for an improved description of SOM quality.

In this work, a new model of SOM decomposition is developed, calibrated and validated. The model is based on a novel implementation of SOM quality by ^{13}C -CPMAS NMR, to purposely overcome the limitations of C/N as single SOM quality indicator and to explore the effects of SOM interactions with soil mineral constituents.

11.2 Model Description

11.2.1 Concept

The SOMDY model presented here has been conceived to represent soil organic matter dynamics taking into account the following major issues:

- Improved definition of chemical composition of SOM
- Assessment of SOM physical structure
- Chemical and physical protection effects on mineralization processes

- Microbial turnover and chemical evolution of SOM during the decomposition
- Impact of agricultural practices on SOM dynamics

Then, the general structure and logic of SOMDY model is represented in Fig. 11.1. The model is modular and developed according to a system dynamic approach. It has been implemented by the Simile software (Muetzelfeldt and Massheder 2003) and it can be downloaded at <http://www.ecoap.unina.it>.

SOMDY is integrated with other models to handle environmental interactions and, specifically, water balance. This is done by interfacing Nasa Casa model (Potter et al. 1993) with water-dependent parameters in SOMDY. The initial requirements to run the integrated model are the definition of soil texture (% of sand, silt, and clay particles), bulk density, adsorbing mineral surface area, and the initial content and chemical composition of soil organic matter. Climatic data (min, max and average temperature, precipitation) are provided at run time by either hourly or daily time steps.

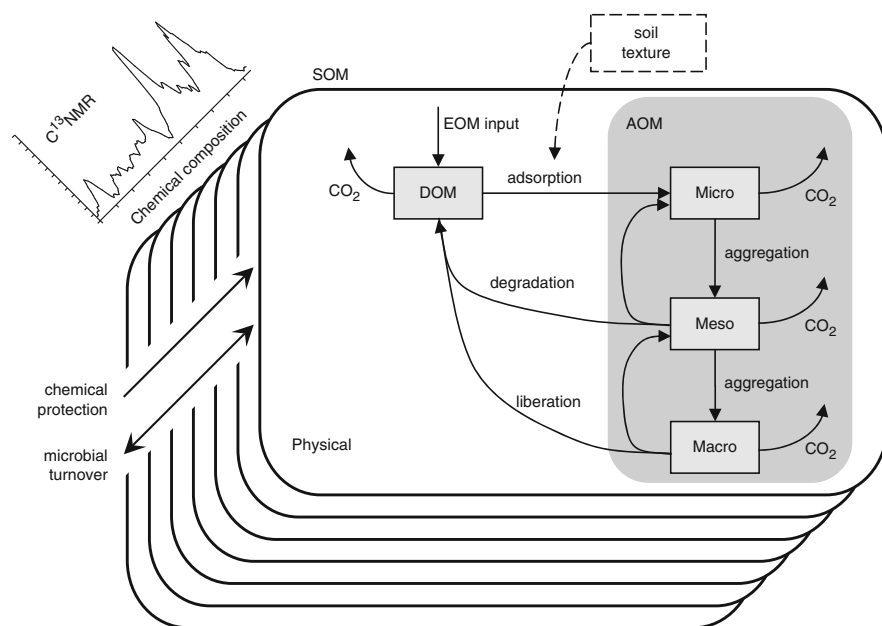


Fig. 11.1 Schematic representation of the logic behind the SOMDY model. Soil organic matter (SOM) is described in terms of its chemical composition (different spectral regions in solid-state ^{13}C NMR spectra correspond to model layers) and physical structure of mineral/organic aggregation status (*DOM* dissolved organic matter; *AOM* aggregated organic matter). Mineralization produces CO_2 emissions with rates depending on both chemical and physical characteristics (see text for details). Microbial turnover is accounted by the change in chemical composition during mineralization processes. The impact of agricultural practices (not shown in figure) is considered to affect liberation and degradation of larger-sized soil aggregates

11.2.2 SOM Chemical Composition and Physical Aggregation

In the model, soil organic matter is a state variable represented by the sum of dissolved (DOM) and aggregated organic matter (AOM). Addition of exogenous organic matter (EOM) is also taken into account with external inputs added to DOM and partitioned according to their specific chemical composition.

Solid-state ^{13}C NMR spectroscopy has been used to assess the chemical composition of organic matter in litter decomposition studies, with different classes of organic-C compounds related to specific NMR spectral regions (Spaccini et al. 2000; Almendros et al. 2000; Kögel-Knabner 2002). In the frame of the SOMDY model, seven resonance regions have been considered, as reported by previous reference studies (Lorenz et al. 2000; Kögel-Knabner 2002; Mathers et al. 2007; Pane et al. 2011): 0–45 ppm = alkyl C, 46–60 ppm = methoxyl and N-alkyl C, 61–90 ppm = O-alkyl C, 91–110 ppm = di-O-alkyl C, 111–140 ppm = H- and C-substituted aromatic C, 141–160 ppm = O-substituted aromatic C (phenolic and O-aryl C), 161–190 ppm = carboxyl C. For calibration purposes, within each wide reference region, a restricted sequence of signals was selected by choosing those most correlated with litter decay rate. Then, the following ranges have been used and referred to the different SOMDY model layers (Fig. 11.1) to represent SOM quality: 10–19, 53–57, 70–75, 103–106, 132–136, 149–153, 175–180 ppm.

The mathematical formulation of such representation of organic matter quality in the model is then the following:

$$\text{SOM} = \sum_{i=1}^7 (\text{DOM} + \text{AOM})_i \quad (11.1)$$

where $i = 1, \dots, 7$, is the chemical class index.

The physical aggregation of organic matter is represented in the model by either dissolved organic materials (DOM) or three different dimensional classes of aggregates: Micro (micro-aggregates, particle diameter $<0.25 \mu\text{m}$), Meso (meso-aggregates, between 0.25 and $1 \mu\text{m}$), and Macro (macro-aggregates, $>1 \mu\text{m}$) (see gray box in Fig. 11.1).

According to the physical structure of SOM (11.1) then becomes:

$$\text{SOM} = \sum_{i=1}^7 (\text{DOM} + \text{Micro} + \text{Meso} + \text{Macro})_i \quad (11.2)$$

The following processes are considered in the SOM system dynamics:

- Mineral and organic adsorption
- Physical aggregation
- Mineralization
- Microbial turnover
- Agricultural practices

11.2.3 Mineral and Organic Adsorption

The adsorbing surface on which organic compounds are aggregated is a function of both the adsorption surface area of the soil mineral fraction and the residual exposed surface area of neo-formed organic aggregates.

This is implemented in the model by calculating the available mineral adsorbing surface, AS_{mineral} , as the sum of surface area in each textural class (sand, silt, and clay):

$$AS_{\text{mineral}} = \text{sand} \cdot AS_{\text{sand}} + \text{silt} \cdot AS_{\text{silt}} + \text{clay} \cdot AS_{\text{clay}} \quad (11.3)$$

where AS_{sand} , AS_{silt} and AS_{clay} are the texture class-adsorbing parameters.

Then, the rate of DOM adsorption is the product of an adsorption coefficient and the available free mineral surface area.

Additionally, as aggregation proceeds through adsorption of organic molecules on soil particles, the model calculates the new surface adsorption created on the newly formed soil aggregates. Differently from the mineral adsorption surface, such organic adsorption surface cannot be saturated, because aggregation process progressively produces new available binding sites for additional DOM, thereby maintaining a somewhat available adsorption surface. For simplicity, organic matter surface adsorption is modeled considering a spherical geometry for organic C aggregates.

11.2.4 Physical Aggregation

Exogenous organic matter (EOM) is considered in the model as an external input to DOM compartment, originating from either litter decomposition or addition of organic amendments by agricultural practise (e.g., compost). DOM can be mineralized with consequent CO_2 release or adsorbed by the mineral and organic soil components. Newly formed Micro-aggregates can then further aggregate forming larger particles (Meso and Macro aggregates). The controlling factors of the aggregation processes are described below. The aggregation process is reversible, i.e., the model also simulates degradation from macro- to meso-, and from meso- to micro-fractions. During degradation, a fraction of organic compounds, previously trapped into the aggregates, is also released as dissolved matter that may flow back to the DOM compartment.

11.2.5 Mineralization

The process of mineralization in the model is represented separately for each chemical class of organic compounds and varies according to the level of physical

aggregation (e.g., differences among DOM and Micro, Meso, Macro). The CO_2 mineralization flow is a function of different parameters:

- *Mineralization rate.* Decay rate changes according to chemical composition, i.e., model layers have different mineralization rates. These were defined on the basis of an extensive correlation analysis between solid-state ^{13}C NMR spectral regions and observed decay rates of 64 different decomposing litter samples under controlled optimal conditions. In general terms, the model simulates the decomposition of different classes of chemical compounds by calculating the changes in ^{13}C spectral regions. Figure 11.2 shows the comparison between two solid-state ^{13}C NMR spectra, corresponding to fresh and decomposed *Quercus ilex* litter, and the output obtained by model simulation of selected spectral regions. The result highlights the model capacity to predict litter decay and relative general chemical changes under optimal decomposition conditions.
- *Saturation effect on mineralization.* The mineralization process is inversely proportional to the available adsorbing mineral surface area. This reflects the fact that in saturated conditions organic matter is more exposed to microbial attack and easier to mineralize. On the other hand, unsaturated mineral particles strongly adsorb residual organic compounds, which become recalcitrant to desorption in the soil solution and to microbial degradation.
- *Temperature effect on mineralization.* Several reviews describing the temperature effects on mineralization processes are available in the literature (Lloyd and Taylor 1994; Kirschbaum 1995; Rodrigo et al. 1997; Del Grosso et al. 2005). In the SOMDY model, a simple exponential function (as in Roth-C or in CASA, Potter et al. 1993) is applied, in order to relate increasing temperature to a proportional increase of mineralization rate.

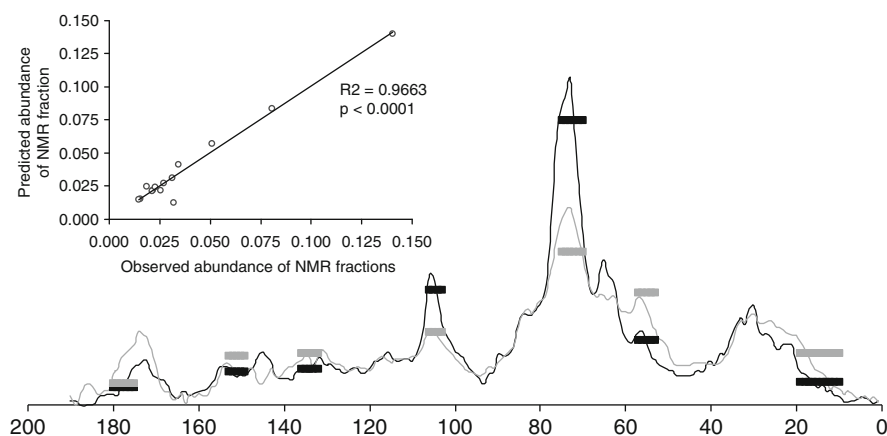


Fig. 11.2 Comparison between real and simulated chemical composition data. Solid-state ^{13}C NMR spectra of *Quercus ilex* litter either fresh (black line) or decomposed for 120 days (gray line). Horizontal bars represent level of simulated spectral regions (initial values: black; changes after 120 days: gray)

- *Water effect on mineralization.* A simple water balance submodel calculates soil water content. The model uses a NASA-CASA-like equation (Potter et al. 1993), as a function of daily precipitation (P , mm), and potential evapo-transpiration (ETP, mm) calculated according to Thornthwaite (1948). The effect of soil water content on the mineralization process is implemented by a sigmoid curve in the water content interval, where no mineralization occurs at a low level of soil water content. Conversely, the increase of water content is proportionally related to an increase in mineralization rate. A negative effect on mineralization rate comes into play at large water content in soil, since the anoxic conditions are to be taken into account.
- *Physical protection.* Besides the effects of chemical composition, and temperature and water content, the OM mineralization rate also depends on a parameter defined as “physical protection,” indicated by h , that is a function of the soil aggregation dimensions ($h_{\text{micro}} < h_{\text{meso}} < h_{\text{macro}}$). This is to say that aggregation generally produces a protection effect vis-à-vis of DOM, but also that micro-aggregates of organic C are more susceptible to decomposition than meso- and macro-aggregates, unless micro-aggregates are not integrated in larger soil aggregates.
- *Chemical protection.* Different chemical compounds are obviously different in their resistance to mineralization and this is reflected by the variable decomposition rates observed in real and simulated regions of NMR spectra (Fig. 11.2). The combined presence of various chemical classes can reduce the mineralization rates of most labile components, since they can become incorporated, and thus protected, into domains composed by more resistant fractions. This phenomenon is represented in the model by a parameter named “chemical protection.” This is a weighing score varying in the $[-1;1]$ range attributed to each chemical layer which reflects the relative resistance to mineralization. A score of 1 indicates a highly protective action against microbial mineralization; zero is given to compounds unaffected by chemical protection, -1 means an enhancement of mineralization due to easily decomposable compounds. The total chemical protection is then calculated by summing up the contributions of all chemical layers. By this procedure, the model shows a considerable effect on OM mineralization rates, thereby well integrating the physical aggregation state and the chemical nature of organic C. In fact, aggregates of similar size can show different mineralization rates due to their specific chemical composition that may be different from that of neighboring aggregates. In other words, the presence of highly resistant compounds can act as a protective shell slowing down mineralization of labile compounds, and, vice versa, easily decomposable materials may enhance the decay of recalcitrant fractions of SOM.

11.2.6 Microbial Turnover

The model structure-based chemical differences among layers also provide a conceptual frame for implementing a submodel on microbial turnover. During the

mineralization processes, a percent fraction of the organic C is converted into microbial biomass. The model does not explicitly describe the processes of microbial feeding, growth and reproduction, but simply calculates the total microbial biomass according to a “metabolic ratio” of all mineralization flows. Then, microbial death is implicitly modeled by re-entering the microbial mass in the system through a partitioning related to a reference microbial composition (Kögel-Knabner 2002). In other words, every time the mineralization occurs, the involved microbes are recycled in the DOM compartments (model layers), in coherence with a chemical description of microbial composition, and, thus, the overall organic matter chemical composition is changed in turn.

11.2.7 Agricultural Practices

The SOMDY model can simulate the effects of agricultural practices in three different ways:

1. Organic matter amendment can be taken into account by adding new chemically defined organic matter (EOM) to the system, and distribute it in the proper model layer by a simple partition according to the different organic components of the EOM.
2. Irrigation is handled by direct addition of water to the water-balance submodel.
3. The impact of agronomic treatments is represented by a linear enhancing effect on the physical degradation of soil aggregates, following the soil management operations. The following practices are considered:
 - (a) Mouldboard plowing affects macro-aggregates stability producing a large release of meso-aggregates
 - (b) Weeding deteriorates macro-aggregates producing some release of meso-aggregates
 - (c) Rotary hoeing (tillage) enhances the degradation of both macro- and meso-aggregates, thus releasing meso- and micro-aggregates, respectively

11.3 Calibration

Two different steps were performed to calibrate the model in terms of parameters and functional responses to different environmental conditions. First, a simplified model application without activation of the physical aggregation module was run to compare the simulated data of organic matter mineralization rates with those of some experimental observations of decaying organic matter under controlled conditions. This procedure was used to define the optimal mineralization parameters (i.e., the potential maximal rates) and to assess the chemical protection factors, by best fitting simulated results with those observed in NMR spectra.

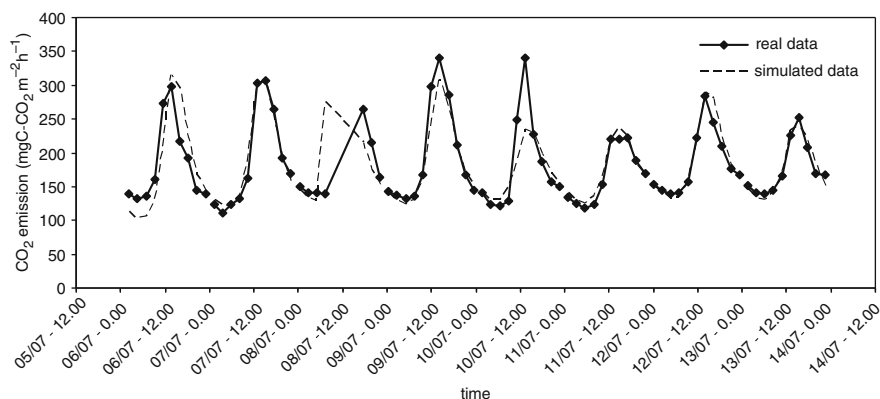


Fig. 11.3 Example of fitting between real and simulated data of CO₂ emissions by mineralization processes of soil organic matter. The simulation refers to one summer week without application of agricultural practices

An example of model performance to simulate changes of organic matter during decomposition in such calibration exercise is reported in Fig. 11.2.

Second, in order to assess the effects of different limiting factors (water, temperature, physical protection), the model was run at hourly time step, comparing simulated CO₂ emissions with field measurements (see Chap. 9 for details). The model parameters were assessed by an iterative procedure of linear programming to optimize the fitting between simulated and observed values. An example of results is presented in Fig. 11.3 showing CO₂ emissions for a period of four summer weeks in an irrigated field at the experimental MESCOSAGR site of Torre Lama, near Napoli (see Chap. 3 for details).

11.4 Simulations

The SOMDY model can be used to simulate soil organic matter dynamics under both theoretical and real scenarios. The model can be run to demonstrate specific processes and effects related to different environmental conditions and agricultural management options.

Figure 11.4 shows an example of a theoretical sequence of applications of different mechanical practices and their average impacts on physical aggregation state and soil carbon loss. It is evident that the occurrence of a plowing or weeding intervention affects, at different intensities, the physical soil structure. This happens because the destruction of Macro aggregates liberates OM and stimulates degradation processes (see Fig. 11.1), thereby reducing the physical protection of organic matter. Such impacts are much stronger in the case of rotary hoeing, and repeated tillage operations end up by completely degrading soil physical structure. As a

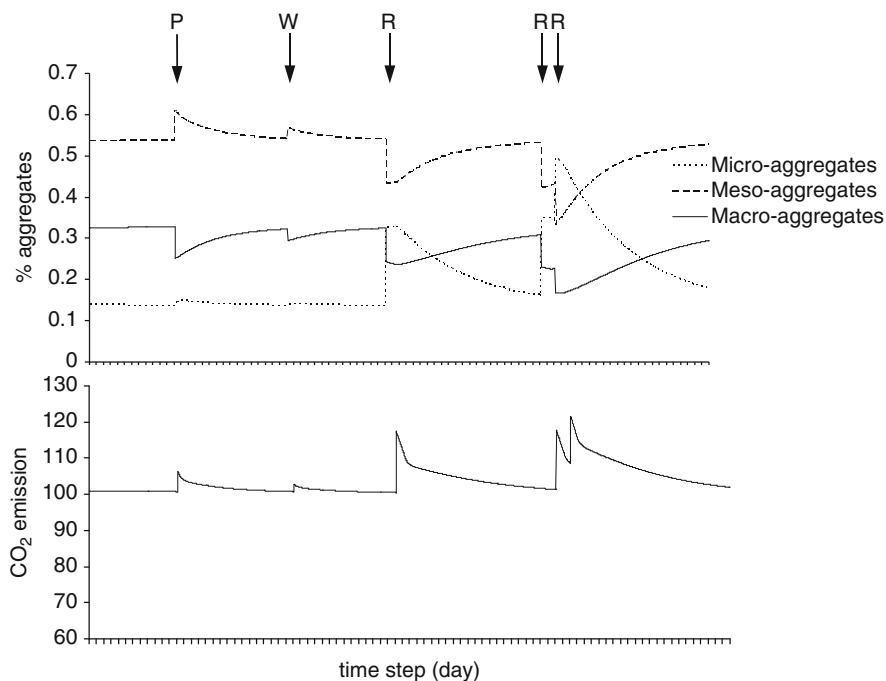


Fig. 11.4 Theoretical demonstration of dynamic behavior for SOMDY model as related to the effects of different agricultural practices on soil organic matter aggregation and associated CO₂ emissions (*P* plowing; *W*: weeding; *R* rotary hoeing)

consequence of a reduction in soil aggregation stability, organic matter is exposed to greater biotic mineralization and larger CO₂ flushes are emitted from soil.

The use of real input data in a simulation exercise allows the reproduction of complex scenarios of agriculture management. The panel of Fig. 11.5 reports the environmental conditions in the year 2007 at the Napoli experimental station (Torre Lama) of the Mescosagr project, for a maize field with traditional management. The model used inputs such as climatic conditions, precipitation, irrigation, and temperature data to calculate the soil–water balance. Further inputs were the initial SOM chemical composition and its physical aggregation state. During a model run, inputs of agricultural practices were applied at the corresponding dates. The result shows the dynamics of physical aggregation states and CO₂ emissions in comparison with real field measurements.

In the case of physical aggregation, the system seems relatively stable during the observation period, despite the oscillations due to temporary impacts of mechanical practices. On the other hand, the model well reproduced CO₂ emissions during the warmer period, whereas the simulated data were slightly overestimated in the colder season.

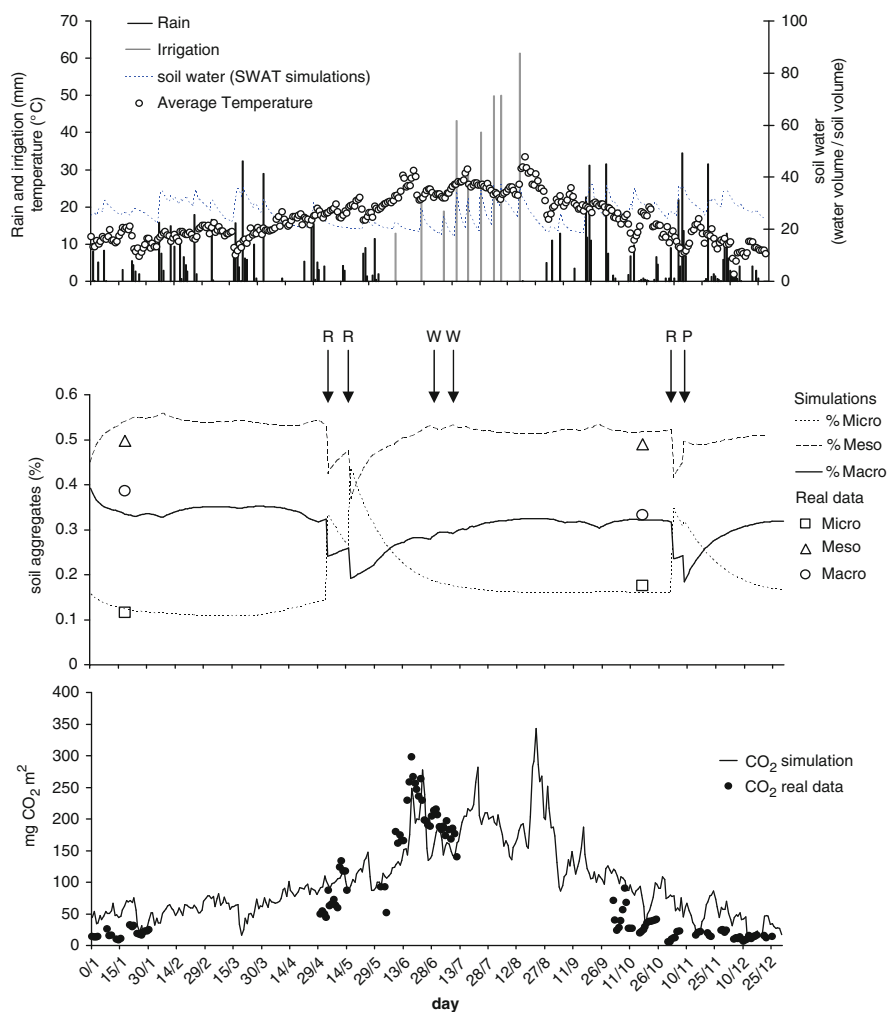


Fig. 11.5 Yearly simulation of SOMDY model with climatic data input (*top*), soil organic matter aggregation and impacts of agricultural practices (*middle* – P: plowing; W weeding; R: rotary hoeing) and CO₂ emissions (*bottom*). See text for details

11.5 Theoretical Scenarios and Future Applications

The SOMDY model presents some innovative features in comparison to other existing published models of decomposition processes of either litter or soil organic matter. In particular, this model is new in its capability to represent the interactions between the chemical nature of organic matter and its physical structure as mineral/organic complexes in the soil. The concepts implemented in SOMDY allow capturing several complex phenomena that may play a major role in controlling

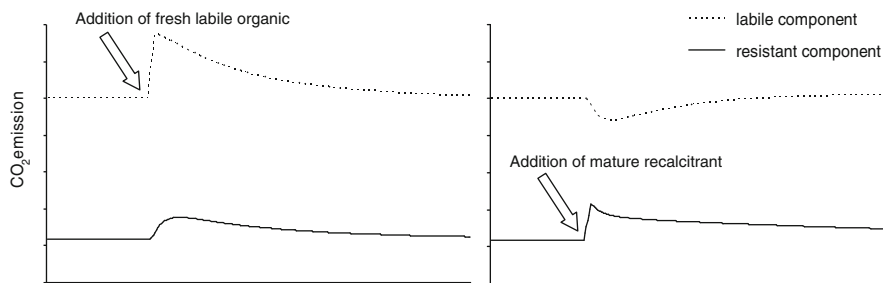


Fig. 11.6 Exercise of theoretical simulation to show the occurrence of priming and protection effects, and related CO₂ release, after addition to soil of organic matter with different chemical composition

soil organic matter dynamics. For instance, it is possible to assess the consequence of organic amendments on carbon sequestration and mineralization processes, as induced by different chemical compositions of organic additions.

Figure 11.6 shows a qualitative example of such emerging complex interactions. Addition of fresh labile organic materials induces a flush of decomposition process with a sudden rise of CO₂ emissions, due to an increased microbial mineralization of the added substrate. Interestingly, also the emissions from resistant components of SOM show a smooth increase due to the well-known “priming” effect (Kuzyako 2002) caused by a greater microbial activity (Fig. 11.6, left-hand side). Furthermore, the addition of a well-humified and/or stabilized EOM such as mature compost induces a relatively slight mineralization increase of the added EOM, but also increases the protection on labile compounds, whose emissions are consequently reduced (Fig. 11.6, right-hand side).

These results demonstrate that our model may well be applied to predict different scenarios of agricultural management, having the capability and suitability to assess their short- and medium-term impacts on SOM and carbon sequestration balance.

Future work will be addressed to improve the model parameterization to fit different environmental conditions and application to long-term predictions aimed to provide an improved environmental sustainability of agricultural managements.

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